CHRONIC TOXICITY SUMMARY

ACROLEIN

(2-Propenal, acraldehyde, allyl aldehyde, acryl aldehyde)

CAS Registry Number: 107-02-8

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.02 μg/m^3** (U.S. EPA-RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Histological changes in nasal epithelium in rats
Hazard index target(s)	Respiratory system; eyes

II. Physical and Chemical Properties (HSDB, 1994)

Molecular formula	C_3H_40
Molecular weight	56.1 g/mol
Specific gravity	0.843 μg 20°C
Boiling point	53° C
Vapor pressure	220 mg Hg μg 20°C
Solubility	Soluble in ethanol, diethyl ether, and up to 20% w/v in water
Description	Colorless liquid/gas
Conversion factor	1 ppm = 2.3 mg/m^3 @ 25° C

III. Major Uses or Sources

Acrolein is principally used as a chemical intermediate in the production of acrylic acid and its esters. Acrolein is used directly as an aquatic herbicide and algicide in irrigation canals, as a microbiocide in oil wells, liquid hydrocarbon fuels, cooling-water towers and water treatment ponds, and as a slimicide in the manufacture of paper (IARC, 1985). Combustion of fossil fuels, tobacco smoke, and pyrolyzed animal and vegetable fats contribute to the environmental prevalence of acrolein (IARC, 1985).

IV. Effects of Human Exposure

Information regarding the toxicity of acrolein to humans is scarce. Acrolein acts primarily as an irritant to the eyes and respiratory tract. The LOAEL for eye irritation is 0.06 ppm (0.14 mg/m^3) acrolein for five minutes (Darley *et al.*, 1960). In this study, 36 healthy human volunteers were

exposed to 0.06 ppm (0.14 mg/m^3) for 5 minutes. Only volunteers without a prior history of chronic upper respiratory or eye problems were included in the study. Subjects wore carbon-filter respirators during exposure, so that only the eyes were exposed to the test mixture. Subjects reported a significant incidence of eye irritation in a questionnaire following the exposure.

V. Effects of Animal Exposure

Male rats were exposed for 6 hours/day, 5 days/week for 62 days to acrolein at concentrations of 0, 0.4, 1.4, and 4.0 ppm (0, 0.92, 3.2, and 9.2 mg/m^3) (Kutzman *et al.*, 1981). Each group of 24 animals was assessed for pulmonary function immediately prior to the end of the experiment. Pulmonary function tests (PFT) included lung volumes, forced respiratory capacity, pulmonary resistance, dynamic compliance, diffusing capacity of carbon monoxide, and multibreath nitrogen washout. At the end of the experiment, animals were killed and histopathological changes in the lung were recorded. Eight additional rats were designated for histopathology and 8 rats were used for reproductive testing only. All analyses were performed post-exposure for 6 days to minimize the acute effects of acrolein. Mortality was high (56%) in rats exposed to 4.0 ppm (9.2 mg/m^3). The observed mortality was due to acute bronchopneumonia in these cases. The animals from this group that survived had reduced body weight. No histological changes were observed in extrarespiratory tissues in any group. There was a concentration-dependent increase in histological changes to the nasal turbinates and rhinitis, beginning at 0.4 ppm. Concentration-dependent damage to the peribronchiolar and bronchiolar regions was also observed. No lung lesions were observed in the 0.4 ppm group. The NOAEL for nasal lesions (squamous epithelial metaplasia and neutrophil infiltration) in this study was 0.4 ppm.

The concentration required for depression of the respiratory rate of mice by 50% (RD_{50}) during 15 minutes of acrolein exposure was estimated as 1.7 ppm (Kane *et al.*, 1979). These authors proposed that the highest concentration suitable for a human air quality standard was 0.001 x RD_{50}, or 0.002 ppm (0.005 mg/m^3).

The pulmonary immunological defense against a bacterial challenge using *Staphylococcus aureus* in mice was dose-dependently impaired following exposure to acrolein at concentrations of 3 and 6 ppm (6.9 and 13.8 mg/m^3) for 8 hours (Astry and Jakab, 1983). In this study, the control exposure was not described.

Leach and associates (1987) found histological changes in pulmonary epithelium and mucosa in rats exposed to 3 ppm acrolein 6 hours/day, 5 days/week, for 3 weeks. In this study, tests for pulmonary and systemic immune function revealed no significant differences between treated and control animals. Similarly, no difference was observed in survival from a bacterial challenge with *Listeria monocytogenes*, although this challenge was intravenous and not intratracheal, and may not have revealed the pulmonary macrophage impairment indicated by Astry and Jakab (1983).

Feron and Kruysse (1977) exposed hamsters (18/gender) to 4 ppm acrolein for 7 hours/day, 5 days/week, for 52 weeks. Mild to moderate histological changes were observed in the upper and

lower respiratory tract. No evidence of toxicity to other organs was apparent at necropsy, although body weight was decreased. Hematology, urinalysis, and serum enzymes were not affected by exposure.

The effects of repeated or continuous exposures of acrolein on rats, guinea pigs, dogs, and monkeys were investigated by Lyon and associates (1970). Animals were exposed to 0.7 or 3.7 ppm (1.6 or 8.5 mg/m^3) acrolein for 8 hours/day, 5 days/week, for 6 weeks, or continuously to 0.21, 0.23, 1.0, or 1.8 ppm (0.5, 2.3, or 4.1 mg/m^3) for 90 days. In these studies, 2 monkeys in the 3.7 ppm intermittent exposure group died within 9 days. Monkeys and dogs salivated excessively during the first week. Squamous metaplasia and basal cell hyperplasia of the trachea was observed in monkeys and dogs; 7 of the 9 monkeys also exhibited bronchiolitis obliterans with squamous metaplasia in the lungs. Bronchopneumonia was noted in the dogs. Inflammation in the lung interstitiae was more prominent in the dogs than in the monkeys. Rats and guinea pigs did not exhibit signs of toxicity when exposed to the 3.7 ppm concentration. Continuous exposure to 1.0 and 1.8 ppm, but not 0.22 ppm acrolein, resulted in salivation and ocular discharge in the monkeys and dogs. Rats and guinea pigs appeared normal at all concentrations. Rats exhibited significant weight loss in the 1.0 and 1.8 ppm groups. Nonspecific inflammatory changes were observed in sections of brain, heart, lung, liver and kidney from all species exposed to 1.8 ppm. The lungs from the dogs showed confluent bronchiopneumonia. Focal histological changes in the bronchiolar region and the spleen were detected at 0.22 ppm in dogs. Nonspecific inflammatory changes at the 0.22 ppm level were apparent in liver, lung, kidney and heart from monkeys, guinea pigs and dogs.

There are no reports of reproductive or developmental toxicity following exposure to acrolein. Kutzman *et al.* (1981) found no significant changes in embryo viability in rats exposed to 4.0 ppm acrolein throughout pregnancy. Similarly, sperm morphology was reportedly not affected at this level.

VI. Derivation of U.S. EPA RfC

Study	Kutzman *et al.*, 1981 (evaluated by U.S. EPA, 1994)
Study population	Fischer-344 rats (24 males per group)
Exposure method	Discontinuous whole-body inhalation exposure of 0, 0.4, 1.4, and 4.0 ppm (0, 0.92, 3.2, and 9.2 mg/m^3)
Critical effects	Histological lesions in the upper airways
LOAEL	0.4 ppm
NOAEL	Not observed (see below)
Exposure continuity	6 hours per day, 5 days/week
Exposure duration	62 days
Average experimental exposure	0.071 ppm (0.16 mg/m^3)
Human equivalent concentration	0.0087 ppm (gas with extrathoracic respiratory effects, RGDR = 0.14 based on MV =

	0.18 m^3/day, SA(ET) = 11.6 cm^2)
LOAEL uncertainty factor	3
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factors	1
Cumulative uncertainty factor	1,000
Reference exposure level	9×10^{-6} ppm (0.009 ppb, 2×10^{-5} mg/m^3, 0.02 $\mu g/m^3$)

The LOAEL for nasal histological changes in mice was considered by U.S. EPA to be 0.4 ppm (0.92 mg/m^3). Only one rat showed slight metaplastic and inflammatory changes, which would be insufficient to demonstrate a statistically significant increase. The potential slight effect, however, was accounted for by only an intermediate 3-fold LOAEL factor.

Significant strengths in the acrolein RfC include (1) the use of a well-conducted study with histopathological analysis, and (2) the demonstration of consistent adverse effects among multiple studies of several species conducted by independent investigators.

Major areas of uncertainty are (1) the lack of adequate human exposure data, (2) limited reproductive toxicity data, (3) the absence of a NOAEL in the major study, and (4) the lack of chronic inhalation exposure studies.

VII. References

Astry CL, and Jakab GJ. 1983. The effects of acrolein exposure on pulmonary antibacterial defenses. Toxicol. Appl. Pharmacol. 67:49-54.

Darley EF, Middleton JT, and Garber MJ. 1960. Plant damage and eye irritation from ozone-hydrocarbon reactions. J. Agric. Food Chem. 8:483-485.

Feron VJ, and Kruysse A. 1977. Effects of exposure to acrolein vapor in hamsters simultaneously treated with benzo(a)pyrene or diethylnitrosamine. J. Toxicol. Environ. Health. 25:119-124.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM version). Micromedex, Inc., Denver, CO (edition expires 7/31/95).

IARC. 1985. International Agency for Research on Cancer. IARC monograph on the evaluation of the carcinogenic risk of chemicals to humans: allyl compounds, aldehydes, epoxides and peroxides. Vol 36, pp. 133-161.

Kane LE, Barrow CS, and Alarie Y. 1979. A short-term test to predict acceptable levels of exposure to airborne sensory irritants. J. Am. Hygiene Assoc. 40:207-229.

Kutzman RS. 1981. A subchronic inhalation study of Fischer 344 rats exposed to 0, 0.4, 1.4, or 4.0 ppm acrolein. Brookhaven National Laboratory, Upton, NY, National Toxicology Program: Interagency Agreement No. 222-Y01-ES-9-0043.

Leach CL, Hatoum NS, Ratajczak HV, and Gerhart JM. 1987. The pathologic and immunologic effects of inhaled acrolein in rats. Toxicol. Lett. 39:189-198.

Lyon JP, Jenkins LJ, Jones RA, Coon RA, and Siegel J. 1970. Repeated and continuous exposure of laboratory animals to acrolein. Toxicol. Appl. Pharmacol. 17:726732.

U.S. Environmental Protection Agency. 1995. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for acrolein.

CHRONIC TOXICITY SUMMARY

ACRYLAMIDE

(2-propenamide; ethylene carboxamide; propenoic acid amide; Optimum; Acrylagel)

CAS Registry Number: 79-06-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.7 µg/m^3**
Oral reference exposure level	**0.0002 mg/kg-day**
Critical effect(s)	Degeneration of the peripheral nervous system in rats
Hazard index target(s)	Nervous system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	C_3H_5NO
Molecular weight	71.08 g/mol
Description	White crystals
Vapor pressure	0.007 mm Hg @ 20°C
Solubility	Soluble in ether; 2155 g/l water @ 30°C; 1550 g/l methanol @ 30°C
Conversion factor	Not applicable

III. Major Uses and Sources

Acrylamide is used as a copolymer or in the synthesis of polymers in such applications as adhesives, fibers, paper sizing, molded parts, water coagulant aids, and textiles.

IV. Effects of Human Exposure

The case history of a 25 year old man occupationally exposed for a period of six months from mixing acrylamide in a closed reactor vessel was described (Davenport *et al.*, 1976). Exposure levels were not quantified in the study. Irritation with red ulceration of the palms and soles developed soon after exposure began, and over the ensuing 3 months he experienced fatigue, anorexia, and weight loss. After 5½ months, the patient developed ataxia, tingling of the hands, slurred speech, and difficulty swallowing. Physical examination showed some muscular weakness and decreased tone, loss of sensation of pain, temperature, position, and vibration in the extremities, and loss of reflexes. Two months of recovery produced no improvement of the

condition. Biopsy of the sural nerve showed axonal swelling and accumulation of neurofilaments with some axonal degeneration.

Symptomatology following exposure of a family of five to acrylamide in well water from a chemical grouting process was described (Igisu *et al.*, 1975). The reported level in the water was 400 ppm acrylamide with a "trace" of dimethylaminopropionitrile. With the exception of a period of about 6 days when the water was used for bathing, the well water was used exclusively for drinking and cooking. The contamination of the well occurred approximately one month before the patients' noted symptoms. Symptoms observed among the exposed persons included runny nose, dizziness, coughing, impaired gait, confusion, poor memory, slurred speech, truncal ataxia, and hallucinations. Within two months all symptoms subsided.

Peripheral neuropathy was characterized in an individual exposed to acrylamide in a mine from handling acrylamide used as a "chemical grout" (Auld and Bedwell, 1967). The patient initially reported a skin rash and, after 7 weeks of working with the material, experienced weakness of the legs, difficulty in walking, and clumsiness of the hands. After 2 months, reported signs included coldness, sweating, and numbness of the extremities. Fourteen weeks after initial exposure, the patient was admitted to the hospital when symptoms worsened. Observed signs included slight inflammation of the wrists, slight impairment of positional and temperature sensation, weakness in the extremities, and difficulty in standing. Fourteen weeks after admission all symptoms subsided.

Six cases of occupational acrylamide poisoning were described (Garland and Patterson, 1967). The onset of symptoms generally occurred 4 to 60 weeks after initial exposure. Frequently reported symptoms included peeling and redness of the hands, weakness and sensory and reflex loss in the extremities, impaired gait, and muscular pain. Electrophysiological effects of acrylamide poisoning were characterized in three of the individuals described in the Garland and Patterson (1967) study (Fullerton, 1969). The only abnormalities observed were slight nerve degeneration in the patient most recently exposed with slight reduction of maximal motor nerve conduction velocity and prolonged distal latencies in muscle response.

V. Effects of Animal Exposure

Fischer 344 rats were treated with acrylamide in drinking water such that the daily dose received was 0, 0.05, 0.2, 1, 5, or 20 mg/kg-day (Burek *et al.*, 1980). The experimental group at each dose level consisted of 10 females at all doses, 26 males in the control group, 29 males in the highest dose group, and 23 males in the remaining dose groups. Of the males, 10 rats were treated for up to 93 days then sacrificed. Ten rats were held for a recovery period of up to 144 days after treatment. No rats in the 0.05 or 0.2 mg/kg-day showed effects in any of the parameters examined in the study including microscopic examination of nervous tissue. Male rats in the 1 mg/kg-day dose group showed minimal signs of nerve degeneration by electron microscopy at the end of the treatment period (female rats were not examined). At 5 mg/kg-day, rats showed more severe peripheral nerve degeneration which was noted at 92 days in males and 93 days in females. This effect was not seen in rats examined 111 days after cessation of treatment. The

most severe effects were noted in the highest dose group (20 mg/kg-day) at the end of the treatment period (92 days for males and 93 days for females). Rats in this dose group showed treatment-related effects including dragging of the hind limbs and decreased body weights. Hematological effects included slightly decreased packed cell volume, red blood cell numbers, and hemoglobin. Serum cholinesterase activity was decreased only in females at the highest dose. Electron microscopic examination of nervous tissue showed demyelination and axonal loss of peripheral nerves and slight degeneration of the spinal cord. Effects believed to be secondary to nerve damage included atrophy of the skeletal muscle and testes and distention of the urinary bladder. Partial or complete reversal of acrylamide-induced damage to nerves occurred after 144 days.

Fischer 344 rats (90/sex/treatment group) were treated with target doses of 0, 0.01, 0.1, 0.5, or 2.0 mg acrylamide/kg-day in drinking water (Johnson *et al.*, 1986). Groups of 10 rats/sex/group were examined for toxicity at 6, 12, and 18 months. Endpoints examined in the study included food and water consumption, body weight, hematological parameters, clinical chemistry of serum, urinalysis, gross pathology, and microscopic tissue histology with special emphasis on evaluation of the nervous system. Mortality among rats of both sexes was increased in the highest dose group by the end of the study. Among male rats in the highest dose group, mean body weight decreased. No consistent pattern of adverse effect from acrylamide treatment was observed in food or water consumption, hematology, clinical chemistry, or urinalysis. Also, no consistent dose-related effects were noted on gross pathology or in organ weights. Degeneration of the tibial nerve was increased over controls in rats in the 2.0 mg/kg-day dose group, beginning minimally at 18 months, but becoming more pronounced after 24 months of exposure. This change, which occurs to some degree during the aging process in untreated rats, was described as swelling of nerve fibers and fragmentation of the myelin and axons with vacuolization of eosinophilic globules. The no adverse effect level (NOAEL) from this study was taken to be 0.5 mg/kg-day.

A lifetime oncogenicity study was conducted, administering acrylamide in drinking water to Fischer 344 rats (1144 total animals) in drinking water for 106 weeks (Friedman *et al.*, 1995). Drinking water content of acrylamide was adjusted such that the dose received was 0, 0.1, 0.5, or 2.0 mg/kg-day for males and 0, 1.0, or 3.0 mg/kg-day for females. Mortality was increased in animals in the high dose groups: in males after the 17th month and in females after the 24th month of the study. The only significant non-cancer effect in the animals was degeneration of the sciatic nerve reported in high-dose male and female rats.

Acrylamide was administered per os at 50 and 20 mg/kg-day to female rats (6 rats in the high- and 4 in the low-dose group) until neurological signs became “severe”, 2-3 weeks in the high dose group and 7-8 weeks in the low dose group (Yoshimura *et al.*, 1992). Histopathological examination of animals in the low dose group showed signs of distal axonopathy with axoplasmal accumulation of neurofilaments in the distal parts of the nerve fibers. Animals in the high dose group also showed distal axonopathy in the long tracts of the spinal cord and peripheral nerves. Further changes observed in the brain included vacuolization of the cerebellar cortex and “condensation of the Purkinje cells” with the accumulation of degenerated organelles in cytoplasmic and dendritic portions of the cells.

Reproductive toxicity and neurotoxicity was examined in Swiss mice exposed to acrylamide in drinking water by a modification of the NTP Reproductive Assessment and Continuous Breeding protocol (Chapin *et al.*, 1995; Heindel *et al.*, 1989). F_0 generation mice (20 mating pairs/ dose group and 40 control mating pairs) were treated with 0, 3, 10, and 30 ppm acrylamide before and after a 14-week cohabitation. Litters produced as a result of the pairings were exposed to the same level as the parental generation and exposed until mating at an average of 74 days of age. Based on water consumption patterns, exposure levels were estimated by the authors to be 0, 0.81, 3.19 and 7.22 mg acrylamide/kg-day for F_0 generation animals and 0, 0.86, 2.9, and 7.7 mg acrylamide/kg-day for F_1 generation mice. Significant effects on reproductive performance included decreased number of live pups per litter, increased early resorptions, increased postimplantation loss, and decreased number of live fetuses in the 30 ppm dose group for the F_0 generation. In the F_0 generation, decreased hindlimb grip strength in males at 30 ppm and forelimb grip strength in females at both 10 and 30 ppm acrylamide were observed. In the F_1 generation, crossover breeding resulted in decreased live pups/litter in the high-dose group, however average live pup weight was significantly increased at the same dose. Male forelimb grip strength was decreased in both the 10 and 30 ppm dose groups. The authors concluded that reproductive toxicity was generally a greater effect than neurotoxicity and was particularly apparent in the F_1 generation animals.

Acrylamide was administered daily by oral gavage to Sprague-Dawley rats from gestational day 6 to lactational day 10 to examine developmental toxicity (Wise *et al.*, 1995). Groups of 12 mated females were treated daily with 0, 5, 10, 15, or 20 mg/kg-day (in 5 ml deionized water / kg body weight). Significantly increased pup mortality occurred in the two highest dose groups, with mortality so high at 20 mg/kg-day that all the animals were sacrificed. Maternal weight gain was reduced significantly in the 3 highest dose groups. Hindlimb splaying occurred among dams in the two highest dose groups. Rat pup body weight was reduced in all dose groups, although the effect was transient in the 5 mg/kg-day dose group. Post-weaning, reduced body weight was observed only among male pups in the 15 mg/kg-day dose group. Among weanling offspring in the 15 mg/kg-day dose group, average horizontal motor activity and auditory startle response were significantly decreased. Adult female offspring (at 11 weeks) in the 15 mg/kg-day dose group showed decreased auditory startle response.

Developmental toxicity of acrylamide was examined in Sprague-Dawley rats and Swiss mice administered acrylamide by oral gavage during organogenesis (Field *et al.*, 1990). Acrylamide was administered at 0, 2.5, 7.5, and 15 mg/kg-day to rats on gestational days 6-20 and 0, 3.0, 15, and 45 mg/kg-day to mice on gestational days 6-17 (29 or 30 animals/dose group). Animals were sacrificed on the last day of exposure and evaluated for both maternal and fetal toxicity. Among rats, maternal gestational weight gain was significantly decreased in both the 7.5 and 15 mg/kg-day dose groups when the gain was adjusted for uterine weight. No dose-related effect on embryo development was observed. Among mice, gravid uterine weight was significantly reduced at both 15 and 45 mg/kg-day. Maternal weight gain was significantly reduced in animals in the 45 mg/kg-day dose group, although the reduction was not significant when gravid uterine weight was subtracted from the total maternal weight. Effects on fetal development included a

reduction in average fetal body weight in the highest dose group. A significant trend toward increased fetuses per litter with an extra rib was also observed.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Burek *et al.*, 1980
Study population	Fischer 344 rats (10 females, 23-29 males/group)
Exposure method	Oral (0, 0.05, 0.2, 1, 5, or 20 mg/kg-day in drinking water)
Critical effects	Degeneration of the peripheral nervous system (axolemma invaginations)
LOAEL	1 mg/kg-day
NOAEL	0.2 mg/kg-day
Exposure duration	92 days continuous in drinking water
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	1,000
Oral reference exposure level	0.0002 mg/kg-day
Inhalation conversion factor	3,500 $\mu g/m^3$ per mg/kg-day
Inhalation reference exposure level	0.7 $\mu g/m^3$

Limited information on levels of acrylamide exposure of humans precludes development of the chronic reference exposure level from the case histories demonstrating toxicity. Furthermore, no data are available from animal studies showing toxic effects of acrylamide from inhalation exposure. For this reason, an oral drinking water study has been selected for development of the chronic REL. The studies by Burek *et al.* (1980) and Johnson *et al.* (1986) showed clear, dose-related adverse effects in rats exposed to acrylamide. Specifically, evidence of progressively more severe degeneration of peripheral nerves with increasing doses of acrylamide was demonstrated by electron microscopy. In the highest dose group in the Burek *et al.* (1980) study, there was behavioral evidence of nerve damage (hind limb dragging). These findings are consistent with the observed toxicity of acrylamide to humans. Davenport *et al.* (1976) and Fullerton (1969) report peripheral nerve degeneration in occupationally exposed individuals. The frequently observed effects of ataxia, sensory loss, and weakness are also consistent with observations made in the animal studies. Burek *et al.* (1980) showed effects with an administered dose of 1 mg/kg-day (LOAEL) and with a NOAEL of 0.2 mg/kg-day, while Johnson *et al.* (1986) found a NOAEL of 0.5 mg/kg-day. The Burek *et al.* (1980) study was selected for the development of the REL because it more thoroughly examined the sensitive endpoints of acrylamide toxicity and clearly established the NOAEL.

The US EPA used the Burek *et al.* (1980) study for the development of the reference dose (RfD). Uncertainty factors applied to the NOAEL included a factor of 10 allowing for the uncertainty in

extrapolating from a subchronic study, a factor of 10 for extrapolating from animals to humans, and a factor of 10 to allow for potentially sensitive human subpopulations. Applying these factors to the NOAEL (0.2 mg/kg-day) results in a reference level of 2 E-4 mg/kg-day. The U.S. EPA methodology has been adopted in deriving the chronic REL. Route-to-route conversion of the RfD results in a REL in inhalation units of 0.7 $\mu g/m^3$. No conversion to parts per million is reported since exposure by inhalation is likely to be in particulate or dust form.

A major strength in the acrylamide REL is the extensive experimental investigations in animals of the acrylamide neurotoxicity that are consistent with neurological impairments observed in exposed humans.

Major uncertainties are (1) the lack of adequate human exposure data and(2) the lack of animal inhalation exposure data.

VII. References

Auld RB, and Bedwell SF. 1967. Peripheral neuropathy with sympathetic overactivity from industrial contact with acrylamide. Can. Med. Assoc. J., 96:652-4.

Burek JD, Albee RR, Beyer JE, Bell TJ, Carreon RM, Morden DC, Wade CE, Hermann EA, and Gorzinski SJ. 1980. Subchronic toxicity of acrylamide administered to rats in the drinking water followed by up to 144 days of recovery. J. Env. Path. Toxicol., 4:157-82.

Chapin RE, Fail PA, George JD, Grizzle TB, Heindel JJ, Harry GJ, Collins BJ, and Teague J. 1995. The reproductive and neural toxicities of acrylamide and three analogues in Swiss mice, evaluated using the continuous breeding protocol. Fundam. Appl. Toxicol., 27:9-24.

Davenport JG, Donald CM, and Sumi SM. 1976. 'Giant axonal neuropathy' caused by industrial chemicals: neurofilamentous axonal masses in man. Neurology, 26:919-23.

Field EA, Price CJ, Sleet RB, Marr MC, Schwetz BA, and Morrissey RE. 1990. Developmental toxicity evaluation of acrylamide in rats and mice. Fundam. Appl. Toxicol., 14:502-12.

Friedman MA, Dulak LH, and Stedham MA. 1995. A lifetime oncogenicity study in rats with acrylamide. Fundam. Appl. Toxicol., 27:95-105.

Fullerton PM. 1969. Electrophysiological and histological observations on peripheral nerves in acrylamide poisoning in man. J. Neurol. Neurosurg Psychiatry, 32:186-92.

Garland TO, and Patterson MWH. 1967. Six cases of acrylamide poisoning. Br. Med. J., 4:134-8.

Heindel JJ, Lamb JC, Chapin RE, Gulati DK, Hope E, George J, Jameson CW, Teague J, and Schwetz BA. 1989. Reproductive toxicity testing by Continuous Breeding. Available from the National Toxicology Program, Research Triangle Park, NC (919) 541-2947.

HSDB. 1996. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 7/31/96).

Igisu H, Goto I, Kawamura Y, Kato M, Izumi K, and Kuroiwa Y. 1975. Acrylamide encephaloneuropathy due to well water pollution. J. Neurol. Neurosurg Psychiatry, 38:581-4.

Johnson KA, Gorzinski SJ, Bodner KM, Campbell RA, Wolf CH, Friedman MA, and Mast RW. 1986. Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. Toxicol. Appl. Pharmacol., 85:154-68.

Wise LD, Gordon LR, Soper KA, Duchai DM, and Morrissey RE. 1995. Developmental neurotoxicity evaluation of acrylamide in Sprague-Dawley rats. Neurotoxicol. Teratol., 17:189-98.

Yoshimura S, Imai K, Saitoh Y, Yamaguchi H, and Ohtaki S. 1992. The same chemicals induce different neurotoxicity when administered in high doses for short term or low doses for long term to rats and dogs. Mol. Chem. Neuropathol., 16:59-84.

CHRONIC TOXICITY SUMMARY

ACRYLIC ACID

(Acroleic acid, ethylene carboxylic acid, propene acid, 2-pronenoic acid, vinyl formic acid)

CAS Registry Number: 79-10-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**1 µg/m^3** (U.S. EPA-RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Respiratory effects (lesions of the olfactory epithelium in mice)
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1994)

Molecular formula	$C_3H_4O_2$
Molecular weight	72.06
Description	Colorless liquid
Vapor pressure	52 mm Hg @ 20° C
Boiling point	141° C
Melting point	14° C
Solubility	Soluble in benzene and acetone; miscible with water, alcohol and several ethers
Conversion factor	2.95 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Acrylic acid is prepared by the hydrolysis of acrylonitrile or by the oxidation of acrolein. Acrylic acid is used as a chemical intermediate in the preparation of various polymers including plastics and paint formulations (HSDB, 1994).

IV. Effects of Human Exposure

No human exposure data were presented by U.S. EPA (1995).

V. Effects of Animal Exposure

Rats and mice were exposed 6 hours per day, 5 days per week for 13 weeks to 5, 25, or 75 ppm acrylic acid (Miller *et al.*, 1981). Dose-dependent lesions (increasing incidence and severity with increasing dose) were observed in the olfactory epithelium of all exposed mice. Rats exhibited the same lesions following exposure to 75 ppm acrylic acid.

Pregnant rats exposed to 225 or 450 ppm acrylic acid 6 hours per day, on days 6-15 of gestation exhibited signs of nasal and eye irritation during exposure (Klimsch and Hellwig, 1991). At necropsy on day 20 of gestation, all exposed dams exhibited degeneration of the olfactory epithelium of the nose with metaplasia of the respiratory epithelium. Assessment of developmental endpoints was not done in this study. Another study reported by the authors in the same citation exposed pregnant rats to 40, 120, or 360 ppm acrylic acid 6 hours per day on days 6-15 of gestation and did not identify any exposure-related developmental toxicity. Maternal toxicity was observed in dams exposed to 120 or 360 ppm acrylic acid as indicated by decreased body weight. Similar findings of maternal toxicity and no developmental toxicity were observed in pregnant rabbits exposed to acrylic acid during the critical periods of gestation (Chun *et al.*, 1993; Neeper-Bradley and Kubena, 1993).

VI. Derivation of U.S. EPA Reference Concentration

Study	Miller *et al.*, 1981 (evaluated by U.S. EPA, 1995)
Study population	B6C3F1 mice (15 per sex per exposure concentration)
Exposure method	Discontinuous whole-body inhalation exposures (5, 25, or 75 ppm)
Critical effects	Lesions in the olfactory epithelium
LOAEL	5 ppm
NOAEL	Not observed
Exposure continuity	6 hours per day, 5 days per week
Exposure duration	13 weeks
Average experimental exposure	0.89 ppm
Human equivalent concentration	0.10 ppm (gas with extrathoracic respiratory effects, RGDR = 0.12 based on MV = 0.04 m^3/day, SA(ET) = 2.9 cm^2)
LOAEL uncertainty factor	3
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.0003 ppm (0.3 ppb, 0.001 mg/m^3 , 1 µg/m^3)

U.S. EPA noted that oral and inhalation toxicity studies of acrylic acid indicate portal-of-entry effects and that specific organ toxicity at other sites is not indicated by the available literature. Kinetic studies of acrylic acid demonstrate the rapid detoxification and limited reactivity within the body; both of which are consistent with low systemic toxicity (U.S. EPA, 1995).

Significant strengths in the acrylic acid RfC include (1) the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis, and (2) the demonstration of a dose-response relationship.

Major areas of uncertainty are (1) the lack of human exposure data, (2) the lack of observation of a NOAEL, and (3) the lack of chronic exposure data.

VII. References

Chun JS, Kubena MF, and Neeper-Bradley TL. 1993. Developmental toxicity dose range-finding study of inhaled acrylic acid vapor in New Zealand white rabbits. Bushy Run Research Center Report No. 92N1007, Union Carbide Co., Export, PA. [cited in IRIS, 1995].

HSDB. 1995. Hazardous Substance Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado.

Klimisch HJ, and Hellwig J. 1991. The prenatal inhalation toxicity of acrylic acid in rats. Fund. Appl. Toxicol. 16:656-666.

Miller RR, Ayres JA, Jersey GC, and McKenna MJ. 1981a. Inhalation toxicity of acrylic acid. Fund. Appl. Toxicol. 1(3):271-277.

Neeper-Bradley TL, and Kubena MF. 1993. Developmental toxicity evaluation of inhaled acrylic acid vapor in New Zealand white rabbits. Bushy Run Research Center Report No. 92N1008, Union Carbide Co., Export, PA. [cited in U.S. EPA, 1995].

U.S. EPA. 1995. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS). Office of Health and Environmental Assessment, Environmental Criteria Assessment Office, Cincinnati, OH.

CHRONIC TOXICOLOGY SUMMARY

ACRYLONITRILE

(acrylonitrile monomer, cyanoethylene, propenenitrile, 2-propenenitrile, VCN, vinyl cyanide)

CAS number: 107-13-1

I. *Chronic Toxicity Summary*

Inhalation reference exposure level	**2 µg/m^3** (U.S. EPA-RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Degeneration and inflammation of nasal epithelium in rats
Hazard index target(s)	Respiratory system

II. *Chemical Property Summary* (HSDB, 1994)

Molecular formula	C_3H_3N
Molecular weight	53.1 g/mol
Description	Clear, colorless to pale yellow liquid (technical grades)
Specific gravity	0.81 @ 25^{o}C/4^{o}C (water = 1)
Boiling point	77.3 ^{o}C
Melting point	-82 ^{o}C
Vapor pressure	100 mm Hg @ 23^{o}C
Solubility	Soluble in isopropanol, ethanol, ether acetone and benzene
Conversion factor	1 ppm = 2.17 mg/m^3 @25 ^{o}C

III. Major Uses or Sources

Acrylonitrile is produced commercially by propylene ammoxidation, in which propylene, ammonia and air are reacted by catalyst in a fluidized bed. Acrylonitrile is used primarily as a co-monomer in the production of acrylic and modacrylic fibers. Uses include the production of plastics, surface coatings, nitrile elastomers, barrier resins, and adhesives. It is also a chemical intermediate in the synthesis of various antioxidants, pharmaceuticals, dyes, and surface active agents. Formerly, acrylonitrile was used as a fumigant for food commodities, flour milling and bakery food processing equipment (HSDB, 1994).

IV. Effects of Human Exposure

Many occupational epidemiology studies have investigated retrospectively the morbidity and mortality of acrylonitrile exposed workers. An increased incidence of lung cancer was associated with acrylonitrile exposure. No significant excess mortality has been observed for any noncarcinogenic endpoint. One early cross-sectional study (Wilson *et al.*, 1948) observed multiple deleterious effects in synthetic rubber manufacturing workers acutely exposed (20 to 45 minutes) to various concentrations of acrylonitrile (16 to 100 ppm, 34.7 to 217 mg/m^3). Mucous membrane irritation, headaches, nausea, feelings of apprehension and nervous irritability were observed in the majority of workers. Other less common symptoms observed included low grade anemia, leukocytosis, kidney irritation and mild jaundice. These effects reportedly subsided with cessation of exposure. Human volunteers exposed for a single 8 hour period to acrylonitrile vapors exhibited no deleterious CNS effects at concentrations ranging from 5.4 to 10.9 mg/m^3 (2.4 to 5.0 ppm) (Jakubowski *et al.*, 1987).

A cross-sectional study (Sakurai *et al.*, 1978) found no statistically significant increases in adverse health effects in chronically exposed workers (minimum 5 years) employed at 6 acrylic fiber factories (n=102 exposed, n=62 matched controls). Mean acrylonitrile levels ranged from 0.1 to 4.2 ppm (0.2 to 9.1 mg/m^3) as determined by personal sampling. Though not statistically significant, slight increases in reddening of the conjunctiva and pharynx were seen in workers from the plant with the highest mean levels (4.2 ppm arithmetic mean). Though problems exist with this study, including small sample size and examiner bias (the medical examiner was not blind to exposure status). The time-weighted average exposure of the group occupationally exposed to 4.2 ppm (9.1 mg/m^3) can be calculated as: TWA = 9.1 mg/m^3 x (10/20)m^3/day x 5 days/7 days = 3 mg/m^3. This level is comparable to the LOAEL(HEC) derived by the U.S. EPA.

V. Effects of Animal Exposure

In the Quast *et al.* (1980) study, Sprague-Dawley rats (100/sex/ concentration) were exposed 6 hours/day, 5 days/week for 2 years to concentrations of 0, 20, or 80 ppm acylonitrile vapors (0, 43, or 174 mg/m^3). A statistically significant increase in mortality was observed in the first year among 80 ppm exposed rats (male and female). Additionally, this 80 ppm exposed group had a significant decrease in mean body weight. Two tissues exhibited treatment-related adverse effects due to acrylonitrile exposure, the nasal respiratory epithelium and the brain.

Proliferative changes in the brain glial cells (i.e., tumors and early proliferation suggestive of tumors) were significantly increased in the 20 ppm (8/100) and 80 ppm (20/100) females versus female controls (0/100), and in the 80 ppm males (22/99) versus male controls (0/100). Noncarcinogenic, extrarespiratory effects were observed in the nasal turbinate epithelium at both exposure concentrations, 20 and 80 ppm, but were statistically significant only in the 80 ppm exposed rats. No treatment-related effects in the olfactory epithelium, trachea, or lower respiratory epithelium were observed at either concentration.

Another study (30/sex/concentration) exposed Sprague-Dawley rats to 0, 5, 10, 20 or 40 ppm acrylonitrile vapor for 5 days/week over 52 weeks, and at 60 ppm for 4 to 7 days, 5 days/week for 104 weeks (Maltoni *et al.*, 1977; Maltoni *et al.*, 1988). Histopathologic examinations were performed, including on lungs, brain, kidney, and liver. No noncarcinogenic effects were reported.

One developmental study exposed rats to acrylonitrile vapors at 0, 40, or 80 ppm (duration adjusted concentrations of 0, 15.4, and 31 mg/m^3, respectively) for 6 hours/day during gestational days 6 to 15. In the 80 ppm exposed group, significant increases in fetal malformations were observed including short tail, missing vertebrae, short trunk, omphalocele and hemivertebra (Murray *et al.*, 1978). No difference in implantations, live fetuses or resorptions were seen in the exposed (40 and 80 ppm) versus the control group. Maternal toxicity was observed as decreased body weight at both exposure levels. This study identified a developmental NOAEL of 15.5 mg/m^3 with a LOAEL of 31 mg/m^3 (with maternal toxicity).

VI. *Derivation of U.S. EPA Reference Concentration*

Study	Quast *et al.*, 1980 (Evaluated by U.S. EPA, 1994)
Study population	Sprague-Dawley rats (100/sex/concentration)
Exposure method	Discontinuous whole-body inhalation exposures (0, 20 or 80 ppm)
Critical effects	Degeneration and inflammation of nasal respiratory epithelium, hyperplasia of mucous secreting cells
LOAEL	20 ppm
NOAEL	Not observed
Exposure continuity	6 hours/day, 5 days/week
Average experimental exposure	3.6 ppm for LOAEL group
Human equivalent concentration	0.9 ppm (gas with extrathoracic respiratory effects, RGDR = 0.25 based on MV = 0.33 m^3/day, SA(ET) = 11.6 cm^2)
Exposure duration	2 years
LOAEL uncertainty factor	3
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factor	10 (lack of inhalation bioassay in second species and lack of reproductive data for inhalation exposures when oral study showed adverse reproductive effects)
Cumulative uncertainty factor	1,000

Inhalation reference exposure level 0.0009 ppm (0.9 ppb; 0.002 mg/m^3; 2 µg/m^3)

Sprague-Dawley rats (100/sex/concentration) were exposed 6 hours/day, 5 days/week for 2 years to 0, 20 or 80 ppm acrylonitrile (0, 43, and 174 mg/m^3 respectively). Significant degenerative and inflammatory changes were observed in the respiratory epithelium of the nasal turbinates at both exposure concentrations (20 and 80 ppm). This treatment-related irritation of the nasal mucosa appeared in the 20 ppm exposed male rats as either epithelial hyperplasia of the nasal turbinates, or as hyperplasia of the mucous secreting cells, and, in the 20 ppm exposed females as either focal inflammation in the nasal turbinates or flattening of the respiratory epithelium of the nasal turbinates. In 80 ppm exposed rats the effects were more severe, including suppurative rhinitis, hyperplasia, focal erosions, and squamous metaplasia of the respiratory epithelium. No treatment related effects in the olfactory epithelium, trachea or lower respiratory system were observed at either concentration. This study identified a LOAEL for pathological alterations in the respiratory epithelium of the extrathoracic region of the respiratory tract of 20 ppm (43 mg/m^3).

Significant strengths in the acrylonitrile REL include (1) the availability of chronic inhalation exposure data from a well-conducted study with histopathological analysis and (2) the demonstration of a dose-response relationship.

Major uncertainties are (1) the lack of adequate human exposure data and (2) the lack of a NOAEL.

VII. References

HSDB. 1994. Hazardous Substance Data Bank. Micromedex Inc. Vol. 20.

Jakubowski M, Linhart I, Pielas G, and Kopecky. 1987. 2-Cyanoethylmercapturic acid (CEMA) in the urine as a possible indicator of exposure to acrylonitrile. Br. J. Ind. Med. 44:834- 840.

Maltoni C, Ciliberit A, and Di Maio V. 1977. Carcinogenicity bioassays on rats of acrylonitrile administered by inhalation and by ingestion. Med. Lav. 68(6):401-411.

Maltoni C, Ciliberti A, Cotti G, and Perino G. 1988. Long-term carcinogenicity bioassays on acrylonitrile administered by inhalation and by ingestion to Sprague-Dawley rats. Ann. NY Acad. Sci. 534:179-202.

Murray FJ, Schwetz BA, Nitschke KD, John JA, Norris JM, and Gehring PJ. 1978. Teratogenicity of acrylonitrile given to rats by gavage or by inhalation. Food Cosmet. Toxicol. 16(6):547-52.

Quast JF, Schwetz DJ, Balmer MF, Gunshow TS, Park CN, and McKenna MJ. 1980. A two-year toxicity and oncogenicity study with acrylonitrile following inhalation exposure of rats. Dow Chemical Co., Toxicology Research Laboratory, Midland, MI.

Sakurai H, Onodera T, Utsunomiya T, Minakuchi H, Iwai H, and Matsumura H. 1978. Health effects of acrylonitrile in acrylic fibre factories. Br. J. Ind. Med. 35:219-225.

U.S. EPA 1994. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) Database. Reference concentration (RfC) for Acrylonitrile.

Wilson RH, Hough GV, McCormick WE. 1948. Medical problems encountered in the manufacture of American-made rubber. Industrial Medicine 17:199-207.

CHRONIC TOXICITY SUMMARY

ALLYL CHLORIDE

(1-chloro-2-propene; 3-chloro-1-propene; 2-propenyl chloride; 3-chloroprene; 3-chloropropene; 3-chloropropylene; chlorallylene; chloro-2-propene)

CAS Registry Number.: 107-05-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**1 µg/m^3** (U.S. EPA-RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	PNS effects (peripheral neurotoxicity including weakness of extremities, lurching motion, unsteady gait, paralysis; degeneration of nerve fibers) in rabbits, rats, and cats
Hazard index target(s)	Nervous system

II. Chemical Property Summary (ACGIH, 1994)

Molecular formula	C_3H_5Cl
Molecular weight	76.53 g/mol
Description	Colorless to yellowish brown or red liquid
Vapor pressure	368 mm Hg @ 25 ^oC
Solubility	Miscible with alcohol, chloroform, ether, petrol ether
Conversion factor	3.1 µg/m^3 per ppb at 25ºC

III. Major Uses or Sources

Allyl chloride is required in the synthesis of a variety of industrially important chemicals including epichlorohydrin; sodium allyl sulphonate; a series of allyl amines and quaternary ammonium salts; allyl esters; and a variety of alcohols, phenols and polyols (HSDB, 1994). Additionally, the synthesis of thermosetting resins used in the manufacture of varnishes, plastics, and adhesives require allyl chloride. Various pharmaceuticals utilize allyl chloride as a chemical intermediate, ranging from diuretics to barbiturates and hypnotic agents, such as aprobarbital, butalbital, methohexital sodium, secobarbital, talbutal and thiamyl sodium.

IV. Effects of Human Exposure

Three occupational studies have associated chronic toxic polyneuropathy in humans with allyl chloride exposure (He *et al.*, 1980; He *et al.*, 1985; He, 1991). The first (He *et al.*, 1980) involved 17 women employed in the manufacture of sodium allyl sulfonate, exposed to unspecified levels of allyl chloride and sodium sulfite for durations ranging from 7 months to 5 years. Clinical signs of polyneuropathy observed included impairment of pain and touch sensation, decreased vibration sensation, slightly decreased posture sensation, weakened muscle strength, loss of ankle reflex, and decreased skin temperature. Electromyography indicated neuropathy in 8 of 13 cases. Nerve conduction velocity in the tibial and perineal nerves was slowed in 7 cases. Rheographic abnormalities observed in 14 of 15 patients. Liver function and other laboratory findings (blood, urine) were normal.

In two later studies similar neurological effects were observed in two other sodium allyl sulfonate factories (He *et al.*, 1985; He, 1991). In factory A allyl chloride exposures ranged from 2.6 to 6,650 mg/m^3 (mean = 138±12 mg/m^3) over 2.5 months to 6 years, based on 68 area samples. In factory B exposures ranged from 0.2 to 25.1 mg/m^3 for 1 to 4.5 years based on 10 area samples. EMG revealed abnormalities in 53% of the factory A workers. Factory B workers displayed mild neuropathy by EMG in 13 of 27 workers. Significant decreases in motor nerve conduction velocity and increased motor distal latency were observed in factory A and factory B workers.

V. Effects of Animal Exposure

Limited animal inhalation data exist regarding the chronic effects of allyl chloride by inhalation. Quast et al (1982a,b) conducted two studies in Fischer rats and B6C3F1 mice. An initial low level study exposed the animals (10/sex/group) to 0, 1, 3, 10 or 20 ppm allyl chloride (0, 3, 9, 30 or 60 mg/m^3) for 6 hours/day, 5 days/week for up to 3 months (interim sacrifice at 1 month). No treatment-related changes were noted in clinical observations, hematology, urinalysis, or organ pathology. Male mice exposed to 20 ppm and sacrificed at 1 month had elevated SGPT and SGOT levels accompanied by glycogen depletion and microscopic multifocal coagulation necrosis in the liver, however these lesions were not observed in rats or mice at 3 months.

The follow-up (Quast *et al.*, 1982b) study exposed the animals (25/sex/group) to higher doses of allyl chloride, 0, 50, 100 or 250 ppm (0, 150, 301 or 752 mg/m^3) for 6 hours/day, 5 days/week for 90 days. No treatment related effects on mortality, clinical observations, body weight, urinalysis, hematology, clinical chemistry, or organ weights were observed. Male rats highly exposed (100 and 250 ppm) and all exposed female rats had increased liver weights, but without accompanying changes in serum liver enzymes or microscopic appearance. No exposure-related changes in the lung were seen. Only microscopic changes in the kidneys were considered related to allyl chloride exposure, increased cytoplasmic granularity and eosinophilic staining of the cortical epithelial cells (100 ppm and 250 ppm rats) and tubule focal collapse and atrophy (250 ppm rats).

An acute lethality inhalation study of allyl chloride exposures in several species reported adverse respiratory tract effects including congestion, hemorrhage, and edema on post-mortem

examination after 2-hour exposures (Boquin *et al.*, 1982). Lack of effects at much lower concentrations in the Quast el al. (1982a,b) studies indicate a lesser sensitivity to respiratory tract effects compared with neurotoxic and renal effects.

The neurological effects of allyl chloride were studied in a small number of rabbits (6/group), cats (1/group at high dose only), and rats (10/group, low dose only) (Boqin *et al.*, 1982). The animals were exposed to 0, 17 or 206 mg/m^3 allyl chloride for 6 hours/day, 6 days/week for either 3 months (high dose) or 5 months (low dose). Clinical observations, hematology, urinalysis, and multiple organ histopathological examination were conducted. Additionally, electromyography (EMG) was conducted in the rabbits only. Neurological changes observed in the rabbits at 206 mg/m^3 included EMG changes indicative of peripheral nerve damage by the end of the first month; and, muscle weakness, lurching motion, and unsteady gait, developing to paralysis in 3 of the 6, by month 2. Post-exposure examination revealed degeneration of peripheral nerve fibers. The single 206 mg/m^3 exposed cat demonstrated muscle weakness and unsteady gait. Other organ changes observed in the rabbits included increase liver and lung weights; sinusoidal and vacuolar liver degeneration; cloudy swelling and fatty degeneration on the renal convoluted tubules; and, thickening of the lung alveolar septa. No evidence of adverse treatment-related effects were found in the rabbits or rats exposed to 17 mg/m^3 of allyl chloride; however, no data were presented in the paper. This study identified a NOAEL for neurological effects of 17 mg/m^3 (5.4 ppm).

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Boquin *et al.*, 1982 (Evaluated by U.S. EPA, 1994)
Study population	Rabbits (6/group), cats (1/group at high dose only), and rats (10/group, low dose only)
Exposure method	Discontinuous whole-body inhalation exposures (0, 17 or 206 mg/m^3 for either 3 months (high dose) or 5 months (low dose).
Critical effects	Peripheral neurotoxicity: weakness of extremities, lurching motion, unsteady gait, paralysis; degeneration of nerve fibers
LOAEL	206 mg/m^3
NOAEL	17 mg/m^3
Exposure continuity	6 hours/day, 6 days/week
Average experimental exposure	3.6 mg/m^3 for NOAEL group
Human equivalent concentration	3.6 mg/m^3 for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))

Exposure duration	5 months for NOAEL group
LOAEL uncertainty factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factor	10 (database deficiencies)
Cumulative uncertainty factor	3,000
Inhalation reference exposure level	0.001 mg/m^3 (1 µg/m^3; 0.0004 ppm; 0.4 ppb)

U.S. EPA evaluated this RfC as a having a low level of confidence because of (1) the small number of animals studied; (2) poor reporting of the low dose (NOAEL) results; (3) conflicting data as to hepatic effects; (4) the lack of reproductive toxicity data; and (5) the lack of chronic exposure studies (U.S. EPA, 1994).

VI. References

Boquin L, Shuwei D, Airu Y, Yinlin X, Taibao G, and Tao C. 1982. Studies on the toxicity of allyl chloride. Ecotox. Environ. Safety 6:19-27.

HSDB. 1994. Hazardous Substance Data Bank. Micromedex Inc. Vol. 20.

He F, Shen D, Guo Y, and Lu B. 1980. Toxic polyneuropathy due to chronic allyl chloride intoxication: A clinical and experimental study. Chin. Med. J. 93(3):177-182. As cited in U.S. EPA, 1994.

He F, Lu B, Zhang S, Dong S, Yu A, and Wang B. 1985. Chronic allyl chloride poisoning. An epidemiology, clinical, toxicological and neuropathological study. G. Ital. Med. Lav. 7(1): 5-15. As cited in U.S. EPA, 1994.

He F. 1991. Institute Occupational Medicine, Chinese Academy of Preventative Medicine/WHO Collaborating Centere Occupational Health, Beijing, China. Letter to Daniel Guth, U.S. EPA, Research Triangle Park, NC, September 16. As cited in U.S. EPA, 1994.

Quast JF, Henck JW, Schuetz DJ, and McKenna MJ. 1982a. Allyl chloride - Subchronic Studies. IA - 90-day inhalation study in laboratory rodents (CDF-Fisher 344 rats and B6C3F1 mice). OTS Document #FYI-AX-0782-0199.

Quast JF, Henck JW, Schuetz DJ, Dittenber DA, and McKenna MJ. 1982b. Allyl chloride - Subchronic studies. IB - Results of an inhalation 4-day probe and 90-day subchronic study in laboratory rodents. OTS Document #FYI-AX-0782-0199.

U.S. Department of Health and Human Services. 1990. NIOSH Pocket Guide to Chemical Hazards. DHHS (NIOSH) Publication No. 90-117.

U.S. EPA. 1994. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) Database. Reference concentration (RfC) for Allyl Chloride.

CHRONIC TOXICITY SUMMARY

AMMONIA

(Anhydrous ammonia; aqueous ammonia)

CAS Registry Number: 7664-41-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**100 μg/m^3** (U.S. EPA-RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Pulmonary function tests or subjective symptomatology in workers
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (From HSDB, 1994)

Molecular formula	NH_3
Molecular weight	17.03 g/mol
Description	Colorless liquid
Specific gravity	0.6818 @ 20°C
Boiling point	-33.5° C
Vapor pressure	6460 mm Hg @ 25°C
Solubility	Soluble in water, alcohol, and ether
Conversion factor	1 ppm = 0.71 mg/m^3

III. Major Uses or Sources

This strongly alkaline chemical is widely used in industry as a feed stock for nitrogen-based chemicals such as fertilizers, plastics and explosives (ATSDR, 1990).

IV. Effects of Human Exposures

Comparisons were made between 52 workers and 31 control subjects for pulmonary function (FVC, FEV_1, FEF_{50} and $FEF_{,5}$) and eye, skin and respiratory symptomatology (Holness *et al.*, 1989). Age, height, and pack-years smoked were treated as covariates for the comparisons. The workers were exposed on average for 12.2 years to mean (time-weighted average) ammonia concentrations of 9.2 ppm (6.4 mg/m^3), while controls were exposed to 0.3 ppm (0.21 mg/m^3). No differences in any endpoints were reported between the exposed and control groups.

Groups of human volunteers (4 per group) were exposed to 25, 50, or 100 ppm (0, 17.8, 35.5, or 71 mg/m^3) ammonia 5 days/week for 2, 4, or 6 hours/day, respectively for 6 weeks (Ferguson *et al.*, 1977). Another group of volunteers was exposed to 50 ppm ammonia for 6 hours/day for 6 weeks. Pulmonary function tests (respiration rate, FVC and FEV_1) were measured in addition to subjective complaints of irritation of the eyes and respiratory tract. The difficulty experienced in performing simple cognitive tasks was also measured, as was pulse rate. There were reports of transient irritation of the nose and throat at 50 or 100 ppm.

V. Effects of Animal Exposures

Rats were continuously exposed to rats at 0, 25, 50, 150, or 250 ppm (0, 18, 36, 107, or 179 mg/m^3) ammonia for 7 days prior to intratracheal inoculation with *Mycoplasma pulmonis*, and from 28 to 42 days following *M. pulmonis* exposure (Broderson *et al.*, 1976). All exposures to ammonia resulted in significantly increased severity of rhinitis, otitis media, tracheitis, and pneumonia characteristic of *M. pulmonis* infection. Exposure to 250 ppm ammonia alone resulted in nasal lesions (epithelial thickening and hyperplasia) unlike those seen in *M. pulmonis*-infected rats.

The growth of bacteria in the lungs and nasal passages, and the concentration of serum immunoglobulin were significantly increased in rats exposed to 100 ppm (71 mg/m^3) ammonia over that seen in control rats (Schoeb *et al.*, 1982).

Guinea pigs (10/group) and mice (20/group) were continuously exposed to 20 ppm (14.2 mg/m^3) ammonia for up to 6 weeks (Anderson *et al.*, 1964). Separate groups of 6 guinea pigs and 21 chickens were exposed to 50 ppm and 20 ppm ammonia for up to 6 and 12 weeks, respectively. All species displayed pulmonary edema, congestion, and hemorrhage after 6 weeks exposure, whereas no effects were seen after only 2 weeks. Guinea pigs exposed to 50 ppm ammonia for 6 weeks exhibited enlarged and congested spleens, congested livers and lungs, and pulmonary edema. Chickens exposed to 200 ppm for 17-21 days showed liver congestion and slight clouding of the cornea. Anderson and associates also showed that a 72-hour exposure to 20 ppm ammonia significantly increased the infection rate of chickens exposed to Newcastle disease virus, while the same effect was observed in chickens exposed to 50 ppm for just 48 hours.

VI. Derivation of U.S. EPA RfC

Study	US EPA, 1995; Holness *et al.*, 1989; Broderson *et al.*, 1976
Study population	52 workers; 31 controls
Exposure method	Occupational inhalation
Critical effects	Pulmonary function, eye, skin, and respiratory symptoms of irritation
LOAEL	25 ppm (Broderson *et al.*, 1976)
NOAEL	9.2 ppm (Holness *et al.*, 1989)
Exposure continuity	8 hours/day (10 m^3/day occupational inhalation rate), 5 days/week
Exposure duration	12.2 years
Average occupational exposure	3 ppm for NOAEL group
Human equivalent concentration	3 ppm for NOAEL group
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Modifying factor	3 (database deficiencies)
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.1 ppm (100 ppb; 0.1 mg/m^3; 100 $\mu g/m^3$)

Significant strengths in the ammonia REL include (1) the availability of long-term human inhalation exposure data, and (2) the demonstration of consistent effects in experimentally exposed human volunteers following short-term exposures.

Major areas of uncertainty are (1) the lack of a NOAEL and LOAEL in a single study, (2) a lack of animal data with histopathological analyses, and (3) difficulties in estimated human occupational exposures.

VII. References

ATSDR. 1990. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Ammonia. ATSDR, U.S. Public Health Service: Atlanta, GA.

Anderson DP, Beard CW, and Hanson RP. 1964. The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. Avian. Dis. 8:369379.

Broderson JR, Lindsey JR, and Crawford JE. 1976. The role of environmental ammonia in respiratory mycoplasmosis of rats. Am. J. Pathol. 85(1):115-130.

Ferguson WS, Koch WC, Webster LB, and Gould JR. 1977. Human physiological response and adaptation to ammonia. J. Occup. Med. 19(5):319-326.

HSDB. 1994. Hazardous Substance Database. Tomes Micromedex, Inc., Vol 20, Denver CO.

Holness DL, Purdham JT, and Nethercott JR. 1989. Acute and chronic respiratory effects of occupational exposure to ammonia. Am. Ind. Hyg. Assoc. J. 50(12):646-650.

Schoeb TR, Davidson MK, and Lindsey JR. 1982. Intracage ammonia promotes growth of Mycoplasma pulmonis in the respiratory tract of rats. Infect. Immun. 38:212217.

U.S. EPA. 1995. U. S. Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for ammonia.

Verberk MM. 1977. Effects of ammonia in volunteers. Int. Arch. Occup. Environ. Health. 39:73-81.

CHRONIC TOXICITY SUMMARY

ANILINE

(Aminobenzene; aminophen; aniline oil; kyanol; phenylamine)

CAS Registry Number: 62-53-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**1 µg/m^3** (U.S. EPA-RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Spleen toxicity in rats
Hazard index target(s)	Circulatory system

II. Physical and Chemical Properties (HSDB, 1994)

Molecular formula	C_6H_7N
Molecular weight	93.12 g/mol
Description	Colorless freshly distilled, otherwise a brown oily liquid
Specific gravity	3.22 @ 25 ^oC (air = 1)
Boiling point	363-367 ^oC
Melting point	-6.3 ^oC
Vapor pressure	0.67 atm @ 25 ^oC
Solubility	Miscible in alcohol, benzene, chloroform
Conversion factor	1 ppm = 3.81 mg/m^3 at 25 ^oC

III. Major Uses or Sources

Aniline is a major chemical intermediate for the synthesis of dyes, pigments, resins, solvents, hydroquinones, herbicides and fungicides. Two major isocyanates used in polyurethane production, methylenediphenyl diisocyanate (MDI) and polymethylene polyphenyl isocyanate (polymeric MDI), require aniline for synthesis. Aniline is also used in manufacturing perfumes, varnishes, vulcanized rubber, explosives, and photographic chemicals (HSDB, 1994).

IV. Effects of Human Exposure

Acute aniline poisoning is characterized by methemoglobin formation with resulting cyanosis or blue skin. This formation of methemoglobin interferes with the oxygen-carrying capacity of the blood. The adverse effects of chronic aniline exposure, whether in humans or laboratory animals, also appear to be secondary to this methemoglobinemia induction (as summarized in U.S. EPA, 1995).

An occupational study was conducted on 58 diphenylamine production workers who were exposed to aniline at estimated airborne concentrations of 1.3 to 2.75 mg/m^3. These exposures, which were 0.5 to 1.0 mg/m^3 on a time-weighted average basis, were reportedly associated with an increase in methemoglobin content after one year (Vasilenko *et al.*, 1972). Other effects reported included decreased hemoglobin levels, erythrocyte number, and coagulative factors. However, no data were presented regarding these health related endpoints.

V. Effects of Animal Exposure

Oberst *et al.*(1956) exposed a small number of rats (9), dogs (2), mice (20) and guinea pigs (10) to 5 ppm (19 mg/m^3) of reagent grade aniline vapor for 6 hours/day, 5 days/week, for either 20 or 26 weeks. Analysis of whole blood, serum, body weight, urinalysis (dogs only), and organ pathology (for dogs and few of the smaller animals), found an methemoglobin increase in only the rats (0.6%, no statistical analysis given). No organ pathology in any species tested was attributed to aniline vapors.

Another subchronic study (duPont deNemours, 1982), exposed male rats (16/group) to either 0, 17, 45 or 87 ppm aniline vapors (0, 65, 171, 331 mg/m^3) for 6 hours/day, 5 days/week, for 2 weeks. Methemoglobin levels were elevated in a dose-dependent manner at 87 ppm (4.2 to 23%) and 45 ppm (2.2 to 5.4%), but not at 17 ppm (0 to 2.9%, not statistically different from controls). Other adverse effects seen in these mid- and high-exposed rats included anemia with decreases in RBCs, hemoglobin content, mean hemoglobin concentration, hematocrit, and increases in mean spleen weight. Spleen toxicity was reported in all exposure groups.

The occurrence of non-neoplastic splenic lesions (fibrosis, mesothelial hyperplasia) in animals exposed to aniline is associated with accumulation of hemosiderin deposits thought to be formed secondarily to methemoglobin (Goodman *et al.*, 1984). However, another study (Weinberger *et al.*, 1985) demonstrated a relationship between splenic lesions and development of splenic sarcomas in animals fed methemoglobinemia producing aniline HCl. A similar finding was noted in animals fed aniline-based food coloring C Red No. 9, which does not induce methemoglobin. Methemoglobin formation may not be necessarily precede splenic lesions for all aniline compounds.

No inhalation studies were found investigating the reproductive effects of aniline. However, a CIIT (1981) study examined Fischer 344 rats gavaged with 10, 30, or 100 mg/kg/day aniline HCl on gestational days 7 to 10. No embryotoxicity or teratogenicity was observed at levels of aniline

that caused maternal toxicity. Increases in methemoglobin and altered hematological measures (decrease RBCs and increase in MCV) were observed in dams of the 100 mg/kg/day group. An dose-dependent increase in spleen weight was observed in all treatment groups. In the fetuses of the 100 mg/kg/day group, liver weight and erythrocyte size were elevated over control values. Observation of pups from parturition to postnatal day 60 found an increase in postnatal deaths, from 8% in controls, to 9.6% at 10 ppm, 20.8% at 30 ppm, and 12.5% at 100 ppm, but the cause of death was undetermined.

VI. Derivation of U.S. EPA Reference Concentration (U.S. EPA, 1995)

Study	Oberst, 1956; du Pont de Nemours, 1982
Study population	9 male Wistar rats and 2 dogs for 26 weeks, and 20 female albino mice and 10 guinea pigs for 20 weeks (Oberst, 1956) Male Crl:CD rats (16/group) (du Pont de Nemours, 1982)
Exposure method	Continuous whole body inhalation (5 ppm reagent grade aniline vapor)
Critical effects	No observed adverse effects in Oberst study. Mild spleen toxicity observed in du Pont de Nemours study.
LOAEL	17 ppm (du Pont de Nemours, 1982)
NOAEL	5 ppm (Oberst, 1956)
Exposure continuity	6 hours/day x 5 days/week (both studies)
Average experimental exposure	0.89 ppm for NOAEL group
Human equivalent exposure	0.89 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Exposure duration	20 weeks (rats, dogs) to 26 weeks (mice, guinea pigs) (Oberst, 1956) 2 weeks (du Pont de Nemours, 1982)
LOAEL factor	1
Subchronic uncertainty factor	10
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Modifying factor	3 (lack of reproductive studies)
Cumulative uncertainty factor	3,000
Inhalation reference exposure level (REL)	0.0003 ppm (0.3 ppb, 0.001 mg/m^3, 1 $\mu g/m^3$)

Oberst *et al.* (1956) exposed 9 male Wistar rats and 2 dogs for 26 weeks, and 20 female albino mice and 10 guinea pigs for 20 weeks, to 5 ppm (19 mg/m^3) of reagent grade aniline vapor for 6 hours/day, 5 days/week. Blood analysis indicated an increase in methemoglobin in rats only (0.6 %, no statistics or control level given). No altered organ pathologies were attributed to aniline

exposure, including a lack of spleen toxicity. A free-standing NOAEL of 19 mg/m^3 (5 ppm) was thus identified as a slight increase of methemoglobin was not considered adverse and the spleen toxicity was not observed.

A second subacute inhalation study (duPont deNemours, 1982) was considered by the U.S. EPA (1995) to demonstrate a LOAEL of 17 ppm based on mild spleen toxicity. This 2-week subacute study exposed male Crl:CD rats (16/group) to 0, 17, 45, or 87 ppm (0, 65, 171, 331 mg/m^3) aniline vapors, 6 hours/day, 5 days/week. Methemoglobin levels were not significantly elevated at 17 ppm (0 to 2.9%), but were elevated in a dose-dependent manner at 45 ppm (2.2 to 5.4%) and 87 ppm (4.2 to 23%). Minimal splenic histopathology was noted in the low-dose 17 ppm group. Rats exposed to 45 or 87 ppm displayed anemia with decreases in RBC counts, hemoglobin content, mean corpuscular hemoglobin concentration and hematocrit, with an accompanying increase in mean relative spleen weight. Additional alterations observed in the spleens from the high dose group included erythropoietin foci, reticuloendothelial cell hypertrophy, and hemosiderin deposition. Though duration of this study was only 2 weeks, the critical effects (methemoglobin increase and splenic involvement) were already manifest, demonstrating the possibility of toxic effects at doses below 17 ppm. The U.S. EPA used this LOAEL as support for the free-standing NOAEL of 5 ppm observed by Oberst (1956).

A 10-fold interspecies uncertainty factor is cited by U.S. EPA (1995), in spite of prior use of the HEC extrapolation. This represents the only such example from U.S. EPA and was presented without explanation. The factor is used in this document to maintain consistency with U.S. EPA.

The major strengths of the REL are the observation of a NOAEL in a long term, multiple-species study. The major uncertainties are the lack of human data, the lack of a demonstration of a dose-response relationship, and the use of differing studies to assess a NOAEL and LOAEL.

VII. References

CIIT. 1981. Chemical Industry Institute of Toxicology. Final report: Teratological and postnatal evaluation of aniline hydrochloride in the Fischer 344 rat. Document No. 40-8376093.

duPont deNemours and Company, Inc. 1982. Subacute inhalation toxicity study of aniline in rats. OTS No. 878220240. Fiche No. 0215025.

Goodman DG, Ward JM, and Reichardt WD. 1984. Splenic fibrosis and sarcomas in F344 rats fed diets containing aniline hydrochloride, p-chloroaniline, azobenzene, o-toluidine hydrochloride, 4,4'-sulfonyldianiline, or D and C Red No. 9. J. Natl. Cancer Inst. 73(1):265-273.

HSDB. 1994. Hazardous Substance Data Bank. Micromedex, Denver, CO.

Oberst F, Hackley E, and Comstock C. 1956. Chronic toxicity of aniline vapor (5 ppm) by inhalation. Arch. Ind. Health 13:379-384.

U.S. EPA. 1995. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) Database. Reference concentration (RfC) for Aniline.

Vasilenko NM, Khizhniakova LN, and Zvezdai VI. 1972. Clinical hygienic parallels in the action of aniline of the body. Vrach. Delo. 8:132-134. As cited in U.S. EPA, 1995.

Weinberger MA, Albert RH, and Montgomery SB. 1985. Splenotoxicity associated with splenic sarcomas in rats fed high doses of D and C Red No. 9 or aniline hydrochloride. J. Natl. Cancer Inst. 75(14):681-690.

CHRONIC TOXICITY SUMMARY

ANTIMONY TRIOXIDE

(antimonious oxide, senarmonite, valentinite, antimony white, antimony peroxide)

CAS Registry Number: 1309-64-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.2 μg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Pulmonary toxicity; chronic interstitial inflammation in rats
Hazard index target(s)	Respiratory system

II. Chemical Property Summary

Molecular formula	Sb_2O_3
Molecular weight	169.8 g/mol
Description	White or colorless solid
Vapor pressure	Sublimes at 1550° C
Solubility	Soluble in tartaric acid, acetic acid, hydrochloric acid; very slightly soluble in water
Conversion factor	Not applicable

III. Major Uses and Sources

The most common use of antimony trioxide is as a textile fire retardant (ATSDR, 1992). Antimony trioxide is also used in pigments, vulcanizing agents and compounds of enamel (Winship, 1987). In the glass industry, antimony trioxide is used as a refining agent and as a glass coloring (Ludersdorf *et al.*, 1987).

IV. Effects of Human Exposure

In an occupational study conducted at a facility smelting antimony ore, the prevalence of reported illnesses in male workers exposed to smelting fumes was greater than in workers in other departments of the facility who, presumably, had less exposure (Renes, 1953). Of the men with illnesses, 20% reported dermatitis, 20% rhinitis, 11% laryngitis, and 10% reported tracheitis. Less frequently observed signs of toxicity included bronchiolitis, conjunctivitis, gastritis,

gastroenteritis, pharyngitis, pneumonitis, and nasal septal perforations. An average air concentration of several work areas was reported as 4.7 mg Sb/m^3. The lowest measured Sb concentration in the smelter was 0.40 mg/ m^3. Airborne arsenic was also detected at the work site.

Pneumonitis was observed in chest x-rays of men exposed for 2-12 hours to heavy but unquantified concentrations of antimony smelter fumes (Renes, 1953). Most of the workers exposed complained of nasal irritation and epistaxis, sore throat, hoarseness, burning and redness of the eyes, metallic taste, chest pain, headache and shortness of breath. Weight loss, nausea, vomiting, diarrhea, loss of the sense of smell, and chest tightness were reported less frequently.

In contrast to the previous study, Linch and Sigmund (1975) suggest that no adverse effects were reported following acute exposures to concentrations as great as 10 mg/m^3 Sb_2O_3 during 24 years of operation at one facility.

A cohort of 51 antimony smelter workers employed for 9 - 31 years was investigated by Potkonjak and Pavlovich (1983). Examinations over a 25 year period included chest X-rays, laboratory analysis, pulmonary function tests, and physical examinations. Analysis of airborne dusts revealed 0.82 - 4.72% free silica, 38.73 - 88.86% antimony trioxide, 2.11 - 7.82% antimony pentoxide, and trace amounts of ferric trioxide and arsenic oxide. More than 80% of the particles were < 5 microns in diameter. Workers with duration of employment longer than 9 years exhibited punctate opacities in X-rays, concentrated in the mid-lung region.

V. Effects of Animal Exposure

Newton *et al.* (1994) exposed rats (65/sex/group) to particles with concentrations of 0, 0.06, 0.51, or 4.50 mg Sb_2O_3/m^3, 6 hours/day, 5 days/week for 1 year. These data are also reported by Bio/dynamics (1990). Animals were sacrificed at 6 and 12 months of exposure, and some were kept for 6 or 12 months after the exposure for a follow-up examination. The eyes, kidneys, liver, prostate, spleen, and urinary bladder were examined in all groups. A mild, dose-dependent ocular irritation was observed at 6 months. At the 12-month follow-up, all surviving rats exposed to Sb_2O_3 had conjunctivitis and cataracts (females only), with a significant dose-response trend. A decreased rate of clearance of particle-laden macrophages was observed in the highest dose-group. Particle-laden macrophages were apparent in all dose groups, but no adverse effects were associated with this observation. An in crease in incidence and severity of pulmonary interstitial and granulomatous inflammation was observed in the highest exposure group. The 0.51 mg Sb_2O_3/m^3 group showed an increased incidence of these lesions, but the effect was not significant when severity of the response was considered.

Rats exposed 6 hours per day, 5 days per week for 13 weeks to 0.92 mg/m^3 Sb_2O_3 exhibited proliferation of lung macrophages 28 weeks after the cessation of exposure (Bio/dynamics, 1985). Because of the integral role that macrophages have in the progression of fibrosis, ATSDR (1992) considered proliferation of macrophages to be an adverse health affect and proposed a subchronic LOAEL of 0.92 mg Sb_2O_3/m^3 in rats.

Watt (1983) exposed rats and miniature swine to 0, 1.9, or 5.0 mg Sb_2O_3/m^3 6 hours/day, 5 days/week for 1 year. No exposure-related effects on survival, hematology, or clinical chemistry was noted. Lung weights were increased in both species. Nonneoplastic pulmonary effects were observed in all exposed animals and included focal fibrosis, adenomatous hyperplasia, multinucleated giant cells, cholesterol clefts, pneumonocyte hyperplasia, and pigmented macrophages. The severity of these lesions increased with increasing concentration and duration. The LOAEL for pulmonary effects in rats was 1.9 mg/m^3.

Extensive pneumonitis was observed in guinea pigs exposed initially to 45.4 mg/m^3 Sb_2O_3 for 2 hours/day for 3 weeks and subsequently for 3 hours/day for up to 30 weeks (Dernehl *et al.*, 1945). No other significant pathologic changes were observed

The absorption and retention of antimony following inhalation exposure are primarily a function of solubility and particle size (Newton *et al.*, 1994; Felicetti *et al.*, 1974; Thomas *et al.*, 1973). Clearance of antimony typically is rapid initially, followed by a slower phase. Aerosols generated at lower temperatures (i.e. 100° F) tended to be more water soluble than aerosols generated at higher temperatures, and exposure to these particles resulted in more systemic absorption and retention in the bone. The less soluble and smaller particles generated at higher temperatures were retained longer in the lung.

VI. Derivation of U.S. EPA RfC

Study	Newton *et al.*, 1994
Study population	Fischer 344 rats (65/sex/group)
Exposure method	Discontinuous inhalation
Critical effects	Chronic pulmonary interstitial inflammation
LOAEL	4.50 mg/m^3
NOAEL	0.51 mg/m^3
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	1 year
Average experimental exposure	0.091 mg/m^3 for NOAEL group
Benchmark Concentration (BMC_{10})	0.87 mg/m^3 (0.16 mg/m^3 continuity-weighted exposure)
Human equivalent concentration	0.074 mg/m^3 (particle with pulmonary respiratory effects, RDDR = 0.48)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factor	3 (database deficiencies)
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.0002 mg/m^3 (0.2 $\mu g/m^3$)

Significant strengths in the antimony REL include (1) the availability of chronic inhalation exposure data, (2) a well-conducted study with extensive histopathological analysis, (3) the demonstration of a dose-response relationship, and (4) the demonstration of consistent adverse effects among multiple studies of several species conducted by independent investigators.

A major area of uncertainty is the lack of adequate human exposure data and the lack of data on reproductive and developmental toxicity.

VII. References

ATSDR. 1992. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Antimony. Prepared by Syracuse Research Corporation under subcontract: Clement International Corporation under contract No. 205-88-0608. Prepared for: Agency for Toxic Substances and Disease Registry, US Public Health Service. September 1992.

Bio/dynamics. A three month inhalation toxicity study of antimony trioxide in the rat followed by a recovery period. Prepared by Bio/dynamics, Inc E Millstone, NJ for the Antimony Oxide Industry Association, Washington, DC, 1985 [cited in ATSDR, 1992].

Dernehl CU, Nau CA, and Sweets HH. Animal studies on the toxicity of inhaled antimony trioxide. J Ind Hyg Toxicol 1945;27:256-262.

Felicetti SA, Thomas RG, and RO McClellan. Retention of inhaled antimony-124 in the beagle dog as a function of temperature of aerosol formation. Health Phys 1974;26(6):525-531. {cited in U.S. EPA RfC, 1996]

Ludersorf, R, Fuchs A, Mayer P, Skulsuksai G, and Schake G. Biological assessment of exposure to antimony and lead in the glass producing industry. Int Arch Environ Health 1987;59:469-474.

Linch AL, and Sigmund JM. Antimony trioxide: Industrial hygiene evaluation of a manufacturing operation. Presented before the 16th Annual American Industrial Hygiene Conference, Atlanta, May 16-21, 1976. [cited in NIOSH, 1978].

Newton PE, Bolte HF, Daly IW, *et al.* Subchronic and chronic inhalation toxicity of antimony trioxide in the rat. Fundam Appl Toxicol 1994;22:561-576.

NIOSH. 1978. National Institute for Occupational Safety and Health. Criteria for a recommended standard...occupational exposure to antimony. Cincinnati, OH, 1978.

Potkonjak V, and Pavlovich M. Antimonosis: A particular form of pneumoconiosis. I. Etiology, clinical and X-ray findings. Int Arch Occup Environ Health 1983;51:199-207.

Renes LE. Antimony poisoning in industry. AMA Arch Ind Hyg Occup Med 1953;7:99-108.

Thomas RG, Felicetti SW, Lucchino RV, and McClellan RO. Retention patterns of antimony in mice following inhalation of particles formed at different temperatures. Proc Soc Exp Biol Med 1973;144(2):544-550. [cited in U.S. EPA RfC, 1996].

U.S. EPA. 1996. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) database. Reference concentration (RfC) for antimony trioxide.

Watt WD. Chronic inhalation toxicity of antimony trioxide: Validation of the threshold limit value. Dissertation, Wayne State University, Detroit, MI. Available from Microfilms, International, Ann Arbor, MI. [cited in U.S. EPA-RfC, 1996].

Winship KA. Toxicity of antimony and its compounds. Adv Drug React Pois Rev 1987;2:67-90.

CHRONIC TOXICITY SUMMARY

ARSENIC AND ARSENIC COMPOUNDS

Molecular Formula	Synonyms	Molecular Weight	% As by Weight	CAS Reg. No.
As	Arsenic black, metallic arsenic	74.92	100%	7440-38-2
As_2O_3	Arsenious acid, crude arsenic, white arsenic	197.82	75.7%	1327-53-3
As_2O_5	Arsenic anhydride, arsenic oxide, arsenic oxide anhydride	229.82	41.3%	1303-28-2
$AsH_3Na_2O_4$	Arsenic acid disodium salt, disodium arsenate, sodium arsenate dibasic	185.91	40.3%	7778-43-0

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.03 μg As/m^3**
Oral reference exposure level	**0.0003 mg/kg bw-day** (based on U.S. EPA RfD)
Critical effect(s)	Decreased fetal weight; increased incidences of intrauterine growth retardation and skeletal malformations in mice
Hazard index target(s)	Reproductive system (teratogenicity); circulatory system; nervous system

II. Physical and Chemical Properties (For metallic arsenic except as noted) (from HSDB, 1995, except as noted)

Molecular formula	See above
Molecular weight	See above
Description	As: Yellow, black or gray solid As_2O_3: White solid
Specific gravity	As: 5.727 @ 14°C As_2O_3: 3.74
Boiling point	613° C (sublimes) (ACGIH, 1992)
Melting point	As: 817°C @ 28 atm As_2O_3: 312.3°C
Vapor pressure	1 mm Hg @ 372° C
Solubility	As: soluble in nitric acid, insoluble in water

	Oxides: soluble in water Salts: soluble in water)
Conversion factor	Not applicable

III. Major Uses or Sources

Ore refining processes, including the smelting of copper and lead, are the major sources by which arsenic dust and inorganic arsenic compounds are released (Grayson, 1978). Arsenic trioxide (As_2O_3) is the most commonly produced form of arsenic. As_2O_3 is used as a raw material for the production of other inorganic arsenic compounds, alloys, and organic arsenic compounds.

IV. Effects of Human Exposure

Smelter workers exposed to concentrations of arsenic up to 7 mg As/m^3 showed an increased incidence in nasal septal perforation, rhinopharyngolaryngitis, tracheobronchitis, and pulmonary insufficiency (Lundgren, 1954; as cited in U.S. EPA, 1984).

In a case-control study, copper smelter workers (n=47) exposed to arsenic for 8-40 years (plus 50 unexposed controls matched for age, medical history, and occupation) were examined by electromyography and for nerve conduction velocity in the arms and legs (Blom *et al.*, 1985). The workers were found to have a statistically significant correlation between cumulative exposure to arsenic and reduced nerve conduction velocities in three peripheral nerves (upper and lower extremities). Slightly reduced nerve conduction velocity in 2 or more peripheral nerves was reported as "more common" among arsenic exposed workers. Minor neurological and electromyographic abnormalities were also found among exposed workers. Occupational exposure levels were estimated to be 0.05-0.5 mg As/m^3, with As_2O_3 the predominant chemical form. Except for three arsenic exposed workers who had long-term exposure to lead, exposure to other heavy metals was insignificant.

The smelter workers described by Blom *et al.* (1985) (number of controls reduced to 48) were further examined for prevalence of Raynaud's phenomenon and for vasospastic tendency by measurement of finger systolic pressure at 10°C and/or 15°C relative to that at 30°C (FSP%)(Lagerkvist *et al.*, 1986). The FSP% was found to covary with the duration of exposure to arsenic and the prevalence of Raynaud's phenomenon was significantly increased among exposed workers. Daily arsenic uptake was estimated at less than 300 µg/day and was confirmed with urinary excretion data.

Hyperpigmentation and hyperkeratinization were observed in workers exposed to 0.4-1 mg/m^3 inorganic arsenic for 2 or more years (Perry *et al.*, 1948).

Dermatitis and irritation of the mucous membranes have been observed in arsenic exposed workers (Vallee *et al.*, 1960).

Chronic exposure to arsenic has been associated with decreased birth weight and an increased rate of spontaneous abortion in female smelter workers. However, this association is confounded by the presence of other toxicants in the smelting process, including lead (Nordstrom *et al.*, 1979).

Hepatic fatty infiltration, central necrosis, and cirrhosis were observed in two patients who ingested As_2O_3 (1% in Fowler's solution) for three or more years (Morris *et al.*, 1974). Daily consumption of 0.13 mg As/kg in contaminated well water resulted in the chronic poisoning and death of four children; at autopsy, myocardial infarction and arterial thickening were noted (Zaldívar and Guillier, 1977).

Anemia and leukopenia have been reported in infants ingesting approximately 3.5 mg As/day in contaminated milk over a period of 33 days (Hammamoto, 1955; as cited in ATSDR, 1989).

Premature birth and subsequent neonatal death was reported in a single individual following ingestion of arsenic (Lugo *et al.*, 1969).

V. Effects of Animal Exposure

Changes in host resistance from inhalation exposure to As_2O_3 aerosol were examined in female CD1 mice using a streptococcus infectivity model and an assay for pulmonary bactericidal activity (Aranyi *et al.*, 1985; Aranyi *et al.*, 1981). Mice (100-200/group) were exposed to As_2O_3 aerosol (or filtered air) for 3 hours/day, 5 days/week, for 1, 5 or 20 days. Aerosol exposed and control mice were then combined before challenge with *Streptococcus zoopidemicus* aerosol (4-8 replicate exposures). Statistically significant increases in mortality ($p < 0.05$) were observed in mice exposed once to 271, 496, and 940 μg As/m^3, 5 times to 519 μg As/m^3, and 20 times to 505 μg As/m^3. Multiple exposures at a given exposure level did not correlate with increased mortality, suggesting an adaptation mechanism. Single exposure did, however, show a dose-response for increased mortality with increasing level of arsenic exposure. Bactericidal activity was evaluated by measuring the ratio of viable bacteria count to radioactive count in the lung 3 hours after infection with ^{35}S-labeled *Klebsiella pneumoniae*. A single exposure to 271, 496, and 940 μg As/m^3, but not 123 μg As/m^3, resulted in significantly decreased bactericidal activity. Five exposures to 519 μg As/m^3 and twenty exposures to both 245 and 505 μg As/m^3 resulted in decreased bactericidal activity.

Female albino rats (20/group) were exposed to 0, 1.3, 4.9, or 60.7 μg As_2O_3/m^3 as aerosol continuously for 3 months (Rozenshtein, 1970). Decreased whole blood sulfhydryl group content, histological changes in the brain, bronchi, and liver, changes in conditioned reflexes, and changes in chronaxy ratio were observed in both the high- and mid-dose groups. Among animals in the high dose group, eosinophilia, decreased blood cholinesterase activity, decreased serum sulfhydryl content, and increased blood pyruvic acid were observed. No significant changes were observed in the low-dose group.

Male mice (8-10/group) were exposed to 0, 0.5, 2.0, or 10.0 ppm sodium arsenite in drinking water for 3 weeks followed by a 28 day recovery period (Blakley *et al.*, 1980). The primary immune response of the spleen (as indicated by changes in IgM-production assayed by plaque-formation) was suppressed at all dose levels. The secondary immune response was also suppressed at all dose levels as indicated by a decrease in the number of IgG producing cells.

Male Sprague-Dawley rats (7-28/group) were exposed to 0, 40, 85, or 125 ppm sodium arsenate in drinking water for 6 weeks (Brown *et al.*, 1976). Rats from all arsenic exposed groups showed increased relative kidney weights, decreased renal mitochondrial respiration, and ultrastructural changes to the kidney.

Male ddY mice (number not stated) received 0, 3, or 10 mg As_2O_3/kg/day orally for 14 days and were examined for changes in concentrations of monoamine-related substances in various brain regions and for changes in locomotor activity (Itoh *et al.*, 1990). Locomotor activity was found to be increased in the low-dose group and decreased in the high-dose group. Several monoamine-related compounds were altered in both dose groups in the cerebral cortex, hippocampus, hypothalamus, and corpus striatum.

Male and female Wistar rats pups (7-10/group) were treated from age 2 to 60 days by oral gavage with daily administration of 0 or 5 mg As/kg body weight (as sodium arsenate) (Nagaraja and Desiraju, 1993; Nagaraja and Desiraju, 1994). After 160 days, body weights, brain weights, and food consumption were decreased in the arsenic exposed group. Acetylcholinesterase (AChE) and GAD activity and GABA levels were decreased in the hypothalamus, brain stem, and cerebellum during the exposure period; all but AChE activity returned to normal during the post-exposure period. Changes in operant conditioning were also observed among the exposed animals.

Female Holtzman rats (>5/group) were treated with 0, 100, 500, 1000, 2000, or 5000 ppm As_2O_3 in feed for 15 days (Wagstaff, 1978). Hexibarbitone sleeping time was altered in all arsenic exposed groups. Body weight and feed consumption were decreased among animals in groups exposed to ≥ 500 ppm As_2O_3. Clinical signs of toxicity observed among arsenic exposed animals included roughened hair, diarrhea, and decreased physical activity.

Male Sprague-Dawley rats and C57 black mice (12/group) were treated with 0, 20, 40, or 85 ppm sodium arsenate in drinking water for up to 6 weeks (Woods and Fowler, 1978). Among arsenic exposed rats, heme synthetase activity was decreased in all exposed groups. Among animals exposed to ≥ 40 ppm sodium arsenate, hepatic ALA synthetase activity was decreased and urinary uroporphyrin and coproporphyrin were increased. Among exposed mice, heme synthetase activity was decreased and uroporphyrinogen I synthetase activity was increased in all exposed groups. Among animals exposed to ≥ 40 ppm sodium arsenate, urinary uroporphyrin and coproporphyrin were increased.

Administration of 3.7 mg As_2O_3/kg/day to rhesus monkeys for 12 months did not result in any neurologic change detectable by an EEG (Heywood and Sortwell, 1979). Two of the 7 animals exposed to this concentration died before the conclusion of the 52 week period. Of the surviving

animals, two were retained for a 52 week recovery period after which they were sacrificed and necropsied; no significant changes in organ weights or gross appearance were noted.

Pregnant CFLP mice (8-11 females/group) were exposed to As_2O_3 for 4 hours/day on gestational days 9-12 at concentrations of 0, 0.26, 2.9, or 28.5 mg As_2O_3/m^3 (~0.2, 2.2, and 21.6 mg As/m^3 (Nagymajtényi *et al.*, 1985). A significant decrease in fetal weight was observed in all the dose groups, with a 3, 9, and 29% reduction in average fetal weight with increasing dose groups. Significantly increased fetal malformations were observed only in the highest dose group; delayed ossification was the primary defect.

Rats exposed to 1 μg As_2O_3/m^3 (0.76 μg As/m^3) for 5 months showed increased preimplantation mortality and delayed ossification in fetuses (Kamkin, 1982). Experimental detail was not presented, thus limiting the usefulness of this study.

A significant decrease in spermatozoa motility was observed in male rats following continuous exposure to 32.4 mg As_2O_3/m^3 for 48 hours (Kamil'dzhanov, 1982). Similarly, motility was decreased after 120 hour exposure to 7.95 mg/m^3, 252 hour exposure to 1.45 mg/m^3, and 800 hour exposure to 0.36 mg/m^3.

Male and female Charles River CD mice (10/group) were treated with 0 or 5 ppm arsenite in drinking water continuously through 3 generations (Schroeder and Mitchener, 1971). Endpoints examined included the interval between litters, the age at first litter, the ratio of males to females, the number of runts, stillborn offspring, failures to breed, and congenital abnormalities. The study showed an alteration in the number of small litters in the arsenic exposed group.

Female CD-1 mice (8-15/group) were treated by oral gavage with 0, 20, 40, or 45 mg sodium arsenite/kg on a single day of gestation between days 8 and 15 (Baxley *et al.*, 1981). Maternal mortality, fetal malformations, and increased prenatal death were observed among animals treated with 40 mg sodium arsenite/kg.

Pregnant golden hamsters (>10/group) were treated by oral gavage with a single administration of 0, 20, or 25 mg/kg sodium arsenite on one of gestational days 8-12 (Hood and Harrison, 1982). Prenatal mortality was increased among animals receiving 25 mg/kg on gestational days 8 and 12 and fetal weights were decreased among animals receiving 25 mg/kg on gestational 12. One dam died following administration of 20 mg/kg.

Intravenous injection of radioactive arsenate (V) or arsenite (III) in several rodent species, including mice and hamsters, resulted in accumulation of arsenic in the lumen of the epididymal duct, suggesting that long term exposure of sperm to arsenic may occur *in vivo* following acute exposure (Danielsson *et al.*, 1984).

VI. Derivation of Chronic Reference Exposure Levels

Derivation of Inhalation Chronic Reference Exposure Level

Study	Nagymajtényi *et al.*, 1985
Study population	CFLP mice (8-11/group)
Exposure method	Discontinuous inhalation exposure
Critical effects	Reduction in fetal weight; increased incidences of intrauterine growth retardation and skeletal malformations
LOAEL	200 μg As/m^3
NOAEL	Not observed
Exposure continuity	4 hr/day
Exposure duration	4 days (gestational days 9-12)
Average experimental exposure	33 μg As/m^3 for LOAEL group (200 x 4/24)
Human equivalent concentration	33 μg As/m^3 for LOAEL group (particle with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	10 (since USEPA severity level > 5)
Subchronic uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	1000
Inhalation reference exposure level	0.03 μg As/m^3

Reports of human inhalation exposure to arsenic compounds, primarily epidemiological studies of smelter workers, indicate that adverse health effects occur as a result of chronic exposure. Among the targets of arsenic toxicity are the respiratory system (Lundgren, 1954), the circulatory system (Lagerkvist *et al.*, 1986), the skin (Perry *et al.*, 1948), the nervous system (Blom *et al.*, 1985) and the reproductive system (Nordstrom *et al.*, 1979). Occupational exposure levels which were associated with these effects ranged from 50-7000 μg As/m^3. These epidemiological studies suffer, however, from confounding as a result of potential exposure to other compounds, thus limiting their usefulness in the development of the chronic REL.

Studies in experimental animals show inhalation exposure to arsenic compounds can produce immunological depression of the respiratory system, developmental defects, and histological or biochemical effects on the nervous system and lung, thus providing supportive evidence of the types of toxicity observed in humans. Among the inhalation studies, the lowest adverse effect level (LOAEL) was quite consistent (245 μg As/m^3 for decreased bactericidal activity in mice - Aranyi *et al.*, 1985; 200 μg As/m^3 for decreased fetal weight in mice - Nagymajtényi *et al.*, 1985; 270 μg As/m^3 for decreased sperm motility in rats - Kamil'dzhanov, 1982). A single study showed effects occurred at 4.9 μg As_2O_3/m^3 (Rozenshtein, 1970), however, lack of detail with respect to endpoints and experimental design limits this study's usefulness. A significant dose-

related reduction in fetal weight and increased incidences of intrauterine growth retardation, skeletal malformations, and hepatocellular chromosomal aberrations were observed in mice following maternal inhalation exposure to 200 μg As/m^3 (260 μg As_2O_3/m^3) for 4 hours on gestation days 9, 10, 11, and 12 ($p<0.05$;). The most sensitive effect, decreased fetal weight, was observed at 200 μg As/m^3, and were taken as a LOAEL. Maternal toxicity data were not reported.

Route-to-route conversion of the LOAEL in the key study indicates that this chronic REL should also be protective of adverse effects which have been observed in studies with oral exposures, either in food or drinking water. Since adverse health effects have been reported among workers exposed to levels near 50 μg As/m^3, use of this human data would produce a chronic REL near that derived from the use of the animal data. The chronic REL from animal data should, therefore, be protective of potential adverse health effects from human exposures.

The major strength of the REL is the identification of a LOAEL that is supported by data from other studies. The major uncertainties are the lack of adequate human data, the lack of a NOAEL observation, the lack of comprehensive, long-term, multiple-dose, multiple-species studies, and the marginal significance of the findings in the low dose group in the Nagymajtényi *et al.* (1985) study.

Derivation of U.S. EPA Oral Reference Dose

Study	Tseng *et al.*, 1968; Tseng, 1977
Study population	>40,000 residentially exposed individuals
Exposure method	Drinking water (residential exposures)
Critical effects	Hyperpigmentation, keratosis, and possible vascular complications
LOAEL	0.17 mg/L (0.014 mg/kg-day)
NOAEL	0.009 mg/L (0.0008 mg/kg-day)
Exposure continuity	Not applicable
Exposure duration	Lifetime
Average exposure	0.006 mg/kg-day for LOAEL group
Human equivalent concentration	0.006 mg/kg-day for LOAEL group
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3
Cumulative uncertainty factor	3
Oral reference exposure level	0.0003 mg/kg bw-day

*Conversion Factors: NOAEL was based on an arithmetic mean of 0.009 mg/L in a range of arsenic concentration of 0.001 to 0.017 mg/L. This NOAEL also included estimation of arsenic from food. Since experimental data were missing, arsenic concentrations in sweet potatoes and rice were estimated as 0.002 mg/day. Other assumptions included consumption of 4.5 L water/day and 55 kg bw (Abernathy *et al.*, 1989). NOAEL = [(0.009 mg/L x 4.5 L/day) + 0.002

mg/day] / 55 kg = 0.0008 mg/kg-day. The LOAEL dose was estimated using the same assumptions as the NOAEL starting with an arithmetic mean water concentration from Tseng (1977) of 0.17 mg/L. LOAEL = [(0.17 mg/L x 4.5 L/day) + 0.002 mg/day] / 55 kg = 0.014 mg/kg-day.

The oral REL is the U.S. EPA's oral Reference Dose (RfD) (IRIS, 1996). The data reported in Tseng (1977) show an increased incidence of blackfoot disease that increases with age and dose. Blackfoot disease is a significant adverse effect. The prevalences (males and females combined) at the low dose are 4.6 per 1000 for the 20-39 year group, 10.5 per 1000 for the 40-59 year group, and 20.3 per 1000 for the >60 year group. Moreover, the prevalence of blackfoot disease in each age group increases with increasing dose. However, a recent report indicates that it may not be strictly due to arsenic exposure (Lu, 1990).

The data in Tseng *et al.* (1968) also show increased incidences of hyperpigmentation and keratosis with age. The overall prevalences of hyperpigmentation and keratosis in the exposed groups are 184 and 71 per 1000, respectively. The text states that the incidence increases with dose, but data for the individual doses are not shown. These data show that the skin lesions are the more sensitive endpoint. The low dose in the Tseng (1977) study is considered a LOAEL. The control group described in Tseng *et al.* (1968; Table 3) shows no evidence of skin lesions and presumably blackfoot disease, although this latter point is not explicitly stated. This group is considered a NOAEL. The arithmetic mean of the arsenic concentration in the wells used by the individuals in the NOAEL group is 9 µg/L (range: 1-17 µg/L) (Abernathy *et al.*, 1989). The arithmetic mean of the arsenic concentration in the wells used by the individuals in the LOAEL group is 170 µg/L (Tseng, 1977; Figure 4). Using estimates provided by Abernathy *et al.* (1989), the NOAEL and LOAEL doses for both food and water are as follows: LOAEL - [170 µg/L x 4.5 L/day + 2 µg/day (contribution of food)] x (1/55 kg) = 14 µg/kg/day; NOAEL - [9 µg/L x 4.5 L/day + 2 µg/day (contribution of food)] x (1/55 kg) = 0.8 µg/kg/day. Although the control group contained 2552 individuals, only 957 (approximately 38%) were older than 20, and only 431 (approximately 17%) were older than 40. The incidence of skin lesions increases sharply in individuals above 20; the incidence of blackfoot disease increases sharply in individuals above 40 (Tseng, 1968; Figures 5, 6 and 7).

This study is less powerful than it appears at first glance. However, it is certainly the most powerful study available on arsenic exposure to people. This study shows an increase in skin lesions, 22% (64/296) at the high dose vs. 2.2% (7/318) at the low dose. The average arsenic concentration in the wells at the high dose is 410 mg/L and at the low dose is 5 mg/L (Cebrian *et al.*, 1983; Figure 2 and Table 1) or 7 mg/L (cited in the abstract). The average water consumption is 3.5 L/day for males and 2.5 L/day for females. There were about an equal number of males and females in the study. For the dose estimates given below we therefore assume an average of 3 L/day. No data are given on the arsenic exposure from food or the body weight of the participants (we therefore assume 55 kg). The paper states that exposure times are directly related to chronological age in 75% of the cases. Approximately 35% of the participants in the study are more than 20 years old (Figure 1). Exposure estimates (water only) are: high dose - 410 mg/L x 3 L/day x (1/55 kg) = 22 mg/kg/day; low dose - 5-7 mg/L x 3 L/day x (1/55 kg) = 0.3-0.4 µg/kg/day. The high-dose group shows a clear increase in skin lesions and is

therefore designated a LOAEL. There is some question whether the low dose is a NOAEL or a LOAEL since there is no way of knowing what the incidence of skin lesions would be in a group where the exposure to arsenic is zero. The 2.2% incidence of skin lesions in the low-dose group is higher than that reported in the Tseng *et al.* (1968) control group, but the dose is lower (0.4 vs. 0.8 mg/kg/day). The Southwick *et al.* (1983) study shows a marginally increased incidence of a variety of skin lesions (palmar and plantar keratosis, diffuse palmar or plantar hyperkeratosis, diffuse pigmentation, and arterial insufficiency) in the individuals exposed to arsenic. The incidences are 2.9% (3/105) in the control group and 6.3% (9/144) in the exposed group. There is a slight, but not statistically significant increase in the percent of exposed individuals that have abnormal nerve conduction (8/67 vs. 13/83, or 12% vs. 16% (Southwick *et al.*, 1983; Table 8). The investigators excluded all individuals older than 47 from the nerve conduction portion of the study. These are the individuals most likely to have the longest exposure to arsenic. Although neither the increased incidence of skin lesions nor the increase in abnormal nerve conduction is statistically significant, these effects may be biologically significant because the same abnormalities occur at higher doses in other studies. The number of subjects in this study was insufficient to establish statistical significance. Table 3 (Southwick *et al.*, 1983) shows the annual arsenic exposure from drinking water. No data are given on arsenic exposure from food or the body weight (assume 70 kg). Exposure times are not clearly defined, but are >5 years, and dose groups are ranges of exposure. Exposure estimates (water only) are: dosed group - 152.4 mg/year x 1 year/365 days x (1/70) kg = 6 µg/kg/day; control group - 24.2 mg/year x year/365 days x (1/70) kg = 0.9 µg/kg/day.

Again because there are no data for a group not exposed to arsenic, there is some question if the control group is a NOAEL or a LOAEL. The incidence of skin lesions in this group is about the same as in the low-dose group from the Cebrian *et al.* (1983) study; the incidence of abnormal nerve conduction in the control group is higher than that from the low-dose group in the Hindmarsh *et al.* (1977) study described below. The control dose is comparable to the dose to the control group in the Tseng *et al.* (1968) and Hindmarsh *et al.* (1977) studies. The dosed group may or may not be a LOAEL, since it is does not report statistically significant effects when compared to the control. This study shows an increased incidence of abnormal clinical findings and abnormal electromyographic findings with increasing dose of arsenic (Hindmarsh *et al.*, 1977; Tables III and VI). However, the sample size is extremely small. Percentages of abnormal clinical signs possibly attributed to As were 10, 16, and 40% at the low, mid and high doses, respectively. Abnormal EMG were 0, 17 and 53% in the same three groups. The exact doses are not given in the Hindmarsh *et al.* (1977) paper; however, some well data are reported in Table V. The arithmetic mean of the arsenic concentration in the high-dose and mid-dose wells is 680 and 70 µg/L, respectively. Figure 1 (Hindmarsh *et al.*, 1977) shows that the average arsenic concentration of the low-dose wells is about 25 µg/L. No data are given on arsenic exposure from food. We assume daily water consumption of 2 liters and body weight of 70 kg. Exposure times are not clearly stated. Exposure estimates (water only) are: low - 25 µg/L x 2 L/day x (1/70) kg = 0.7 µg/kg/day; mid - 70 µg/L x 2 L/day x (1/70) kg = 2 µg/kg/day; high - 680 µg/L x 2 L/day x (1/70) kg = 19 µg/kg/day. The low dose is a no-effect level for abnormal EMG findings. However, because there is no information on the background incidence of abnormal clinical findings in a population with zero exposure to arsenic, there is no way of knowing if the low dose is a no-effect level or another marginal effect level for abnormal clinical findings. The

low dose is comparable to the dose received by the control group in the Tseng (1977) and Southwick *et al.* (1983) studies.

The responses at the mid dose do not show a statistically significant increase but are part of a statistically significant trend and are biologically significant. This dose is an equivocal NOAEL/LOAEL. The high dose is a clear LOAEL for both responses. As discussed previously there is no way of knowing whether the low doses in the Cebrian *et al.* (1983), Southwick *et al.* (1983) and Hindmarsh *et al.* (1977) studies are NOAELs for skin lesions and/or abnormal nerve conduction. However, because the next higher dose in the Southwick and Hindmarsh studies only shows marginal effects at doses 3-7 times higher, the Agency feels comfortable in assigning the low doses in these studies as NOAELs. The Tseng (1977) and Tseng *et al.* (1968) studies are therefore considered superior for the purposes of developing an RfD and show a NOAEL for a sensitive endpoint. Even discounting the people <20 years of age, the control group consisted of 957 people that had a lengthy exposure to arsenic with no evidence of skin lesions.

The following is a summary of the defined doses in mg/kg-day from the principal and supporting studies:

1) Tseng (1977):	NOAEL = 0.0008;	LOAEL = 0.014
2) Cebrian *et al.* (1983):	NOAEL = 0.0004;	LOAEL = 0.022
3) Southwick *et al.* (1983):	NOAEL = 0.0009;	LOAEL = none (equivocal effects at 0.006)
4) Hindmarsh *et al.* (1977):	NOAEL = 0.0007;	LOAEL = 0.019 (equivocal effects at 0.002)

There was not a clear consensus among U.S. EPA scientists on the oral RfD. Applying the U.S. EPA's RfD methodology, strong scientific arguments can be made for various values within a factor of 2 or 3 of the currently recommended RfD value, i.e., 0.1 to 0.8 µg/kg/day. It should be noted, however, that the RfD methodology, by definition, yields a number with inherent uncertainty spanning perhaps an order of magnitude. New data that possibly impact on the recommended RfD for arsenic will be evaluated by the U.S. EPA Work Group as it becomes available. Risk managers should recognize the considerable flexibility afforded them in formulating regulatory decisions when uncertainty and lack of clear consensus are taken into account.

The Uncertainty Factor (UF) of 3 is to account for both the lack of data to preclude reproductive toxicity as a critical effect and to account for some uncertainty in whether the NOAEL of the critical study accounts for all sensitive individuals. No modifying factor was used.

U.S. EPA stated its confidence in the oral RfD as: Study - Medium; Data Base - Medium; and RfD - Medium. Confidence in the chosen study is considered medium. An extremely large number of people were included in the assessment (>40,000) but the doses were not well-characterized and other contaminants were present. The supporting human toxicity data base is extensive but somewhat flawed. Problems exist with all of the epidemiological studies. For example, the Tseng studies do not look at potential exposure from food or other source. A similar criticism can be made of the Cebrian *et al.* (1983) study. The U.S. studies are too small

in number to resolve several issues. However, the data base does support the choice of NOAEL. It garners medium confidence. Medium confidence in the RfD follows.

VII. References

ACGIH. 1992. American Conference of Governmental Industrial Hygienists, Inc. Documentation of the threshold limit values and biological exposure indices. Sixth edition. Cincinnati, OH.

Abernathy CO, Marcus W, Chen C, Gibb H, and White P. 1989. Office of Drinking Water, Office of Research and Development, U.S. EPA. Memorandum to O. Cook, Office of Drinking Water, U.S. EPA and P. Preuss, Office of Regulatory Support and Scientific Management, U.S. EPA. Report on Arsenic (As) Work Group Meetings. February 23.

Aranyi C, Bradof JN, Fenters JD, Graham JA, and Miller FJ. 1981. Effects of inhalation of arsenic trioxide aerosols in the pulmonary defenses of mice. In: International Conference. Heavy Metals in the Environment. Commission of the European Communities and the World Health Organization. pp. 450-3.

Aranyi C, Bradof JN, O'Shea WJ, Graham JA, and Miller FJ. 1985. Effects of arsenic trioxide inhalation exposure on pulmonary antibacterial defenses in mice. J Toxicol Environ Health, 15:163-72.

ATSDR. 1989. Agency for Toxic Substances and Disease Registry. US Public Health Service. Toxicological profile for arsenic. Prepared by Life Systems, Inc. under contract No. 68-02-4228.

Baxley MN, Hood RD, Vedel GC, Harrison WP, and Szczech GM. 1981. Prenatal toxicity of orally administered sodium arsenite in mice. Bull Environ Contam Toxicol, 26:749-56.

Blakley BR, Sisodia CS, and Mukkur TK. 1980. The effect of methylmercury, tetraethyl lead and sodium arsenite on the humoral immune response in mice. Toxicol Appl Pharmacol, 52:245-54.

Blom S, Lagerkvist B, and Linderholm H. 1985. Arsenic exposure to smelter workers. Scand J Work Environ Health, 11:265-9.

Brown MM, Rhyne BC, Goyer RA, and Fowler BA. 1976. Intracellular effects of chronic arsenic administration on renal proximal tubule cells. J Toxicol Environ Health, 1:505-14.

Cebrian ME, Albores A, Aguilar M, and Blakely E. 1983. Chronic arsenic poisoning in the north of Mexico. Human Toxicol. 2: 121-133.

Danielsson BRG, Dencker L, Lindgren A, and Tjalve H. 1984. Accumulation of toxic metals in male reproductive organs. Arch Toxicol Suppl, 7:177-80.

Grayson M. (ed.) 1978. Kirk-Othmer Encyclopedia of Chemical Technology. Third ed. John Wiley and Son, New York, NY, pp.247-51.

Hammamoto E. 1955. Infant arsenic poisoning by powdered milk. Japanese Medical Journal, 1649:2-12 [cited in ATSDR, 1989].

Hindmarsh JT, McLetchie OR, Hefferman LPM *et al.* 1977. Electromyrographic abnormalities in chronic environmental arsenicalism. J. Analyt. Toxicol. 1:270-276.

Heywood R, and Sortwell RJ. 1979. Arsenic intoxication in the rhesus monkey. Toxicol Lett, 3:137-44.

Hood RD, and Harrison WP. 1982. Effects of prenatal arsenite exposure in the hamster. Bull Environ Contam Toxicol, 29:671-8.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 7/31/96).
U.S. Environmental Protection Agency. 1994. Integrated Risk Information System (IRIS) Database. Reference concentration (RfC) for Arsine.

IRIS. Integrated Risk Information System. Arsenic, inorganic.

Itoh T, Zhang YF, Murai S, Saito H, Nagahama H, Miyate H, Saito Y, and Abe E. 1990. The effect of arsenic trioxide on brain monoamine metabolism and locomotor activity of mice. Toxicol Lett, 54:345-53.

Kamil'dzhanov AX. 1982. Hygienic basis for the maximum permissible concentration of the arsenic trioxide in the ambient air. Gig Sanit, 2:74-5.

Kamkin AB. 1982. For a revision of the maximum permissible concentration of arsenic trioxide in the ambient air of inhabited areas. Gig Sanit, 1:6-9.

Lagerkvist B, Linderholm H, and Nordberg GG. 1986. Vasospastic tendency and Raynaud's phenomenon in smelter workers exposed to arsenic. Environ Res, 39:465-74.

Lugo G, Cassady G, and Palmisano P. 1969. Acute maternal arsenic intoxication with neonatal death. Am J Dis Child, 117:328-30.

Lundgren KD. 1954. [Damage to respiratory organs in workers in a smelting plant]. Nor Hyg Tidskr, 3:66-82 [cited in U.S. EPA, 1984].

Morris JS, Schmid M, Newman S, Scheuer PJ, and Sherlock S. 1974. Arsenic and noncirrhotic portal hypertension. Gastroenterology, 64:86-94.

Nagaraja TN, and Desiraju T. 1994. Effects on operant learning and brain acetylcholine esterase activity in rats following chronic inorganic arsenic intake. Hum Exp Toxicol, 13:353-6.

Nagaraja TN, and Desiraju T. 1993. Regional alterations in the levels of brain biogenic amines, glutamate, GABA, and GAD activity due to chronic consumption of inorganic arsenic in developing and adult rats. Bull Environ Contam Toxicol, 50:100-7.

Nagymajtényi L, Selypes A, and Berencsi G. 1985. Chromosomal aberrations and fetotoxic effects of atmospheric arsenic exposure in mice. J Appl Toxicol, 5:61-3.

Nordstrom S, Beckman L, and Nordenson I. 1979. Occupational and environmental risks in and around a smelter in northern Sweden. V. Spontaneous abortion among female employees and decreased birth weight in their offspring. Hereditas, 90:291-6.

Perry K, Bowler RG, Buckell HM, Druett HA, and Scilling RSF. 1948. Studies in the incidence of cancer in a factory handling inorganic compounds of arsenic. II. Clinical and environmental investigations. Br J Ind Med, 5:6-15.

Rozenshtein IS. 1970. [Sanitary toxicological assessment of low concentrations of arsenic trioxide in the atmosphere]. Gig Sanit, 35:16-21.

Schroeder HA, and Mitchener M. 1971. Toxic effects of trace elements on reproduction of mice and rats. Arch Environ Health, 23:102-6.

Southwick JW, Western AE, Beck MM, *et al.* 1983. An epidemiological study of arsenic in drinking water in Millard County, Utah. In: Arsenic: Industrial, Biomedical, Environmental Perpsectives, W.H. Lederer and R.J. Fensterheim, Ed. Van Nostrand Reinhold Co., New York. p. 210-225.

Tseng WP. 1977. Effects and dose-response relationships of skin cancer and blackfoot disease with arsenic. Environ. Health Perspect. 19: 109-119.

Tseng WP, Chu HM, How SW, Fong JM, Lin CS, and Yeh S. 1968. Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. J. Natl. Cancer Inst. 40: 453-463.

U.S. EPA 1984. United States Environmental Protection Agency. Health Assessment Document for Inorganic Arsenic. U.S. EPA Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment, Research Triangle Park, NC. EPA-600/8-83-012F, Final Report. pp. 5-20.

Vallee BL, Ulmer DD, and Wacker WEC. 1960. Arsenic toxicology and biochemistry. Arch Ind Health, 21:132-51.

Wagstaff DJ. 1978. Alteration of hepatic detoxification enzyme activity by dietary arsenic trioxide. Food Cosmet Toxicol, 16:423-6.

Woods JS, and Fowler BA. 1978. Altered regulation of mammalian hepatic heme biosynthesis and urinary porphyrin excretion during prolonged exposure to sodium arsenate. Toxicol Appl Pharmacol, 43:361-71.

Zaldívar R, and Guillier A. 1977. Environmental and clinical investigations on endemic chronic arsenic poisoning in infants and children. Zentralbl Bakt Hyg, 1 Abt Orig B, 165:226-34.

CHRONIC TOXICITY SUMMARY

ARSINE

(Arsenic hydride, arsenic trihydride, hydrogen arsenide)

CAS Registry Number: 7784-42-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.05 µg/m^3** (U.S. EPA-RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Blood effect (hemolysis, abnormal red blood cell morphology) in mice Spleen effect (increased weight) in mice
Hazard index target(s)	Circulatory system

II. Chemical Property Summary (HSDB, 1994)

Molecular formula	AsH_3
Molecular weight	77.93
Description	Colorless vapor
Boiling point	-55° C
Melting point	-117° C
Specific gravity	2.695 @ 25 ^oC (air = 1)
Vapor pressure	11,000 torr @ 25° C
Solubility	Soluble in chloroform and benzene, slightly soluble in water, ethyl alcohol and in alkalis
Conversion factor	3.19 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Processes such as smelting, galvanizing, soldering and etching, that require the treatment of metal with strong acids are possible sources of arsine gas. Acid treatment of metals contaminated with arsenic can result in the release of arsine gas. Arsine is utilized to provide arsenic as a dopant in the semiconductor industry. Combustion of fossil fuels may produce arsine gas (HSDB, 1994).

IV. Effects of Human Exposure

Arsine gas is a potent hemolytic agent, capable of inducing hemoglobinuria, jaundice, and hemolytic anemia in exposed humans, usually following acute occupational exposure. Arsine induced hemolysis may progress to oliguric renal failure and death in severe cases; however, hemolytic anemia is the most consistent clinical finding in humans. Reported symptoms of acute and subchronic exposures are abdominal pain, hematuria, jaundice, headache, malaise, and gastrointestinal distress. The observed hemolytic effects in humans are consistent with effects seen in arsine exposed animals, including increased hemoglobin concentrations; reticulocytosis; leukocytosis; and, altered RBC morphology characterized by basophilic stippling, anisocytosis, poikilocytosis, red-cell fragments, and ghost cells (U.S. EPA, 1994).

V. Effects of Animal Exposure

Blair and colleagues (1990a) exposed Fischer rats, B6C3F1 mice and Syrian Golden hamsters to arsine. Exposure concentrations were 0, 0.025, 0.5, and 2.5 ppm arsine for a 13-week study, and 0, 0.5, 2.5, and 5 ppm for 14 day and 4 week studies. Blood and tissues samples were collected post-exposure at 1 and 3 days (14 days and 4 week exposure groups), or 3 and 4 days (13 week exposure groups). Interim hematologic samples were only collected for rats in the 13 week exposure group (1, 3, and 11.5 days). Histopathology and packed cell volumes (PCV) determinations were performed on all animal species. Amino levulinic acid dehydratase (ALAD) activity in red blood cells (RBCs) was assayed in all species to determine arsine's effect on the heme synthetic pathway. Arsine treatment-related lesions were seen only in the spleen (all species), liver (mice only), and bone marrow (rats only). No clinical effects were reported in any of the species. Enlarged spleens and significantly increased relative spleen weight were observed in the mid- and high dose groups (0.5 and 2.5 ppm) in rats and hamsters, and in the high dose group in mice (2.5 ppm). Increased hemosiderosis, extramedullary hematopoiesis in the spleen and bone marrow, and hyperplasia of bone marrow were present in the high dose (2.5 ppm) rats. Decreased red blood cell (RBC) counts, Hgb concentrations, and HCTs were present in blood at 80 or 81 days in all exposed female and mid/high dosed male rats. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), platelet count, and ALAD activity were increased in the mid- and/or high-concentration groups. Reduced PCV also occurred in these groups. Mice and hamsters also displayed significantly reduced PCV and increased ALAD activity at these doses. Additionally, mice displayed intracanalicular bile stasis in the liver of 2.5 ppm exposed males and females, with increased relative liver weight in the 2.5 ppm exposed males mice.

A second study by Blair and associates (1990b) exposed B6C3F1 mice to arsine under the same conditions, but with interim blood samples at 5, 15 and 90 days of exposure, found similar altered hematological parameters in a concentration dependent manner. Decreased RBC counts, Hgb concentrations, and HCTs; increased MCVs and MCHs; and, elevated methemoglobin and absolute reticulocyte counts were observed in the high exposure group. Morphological evaluation of RBCs identified polychromasia, anisocytosis, poikilocytosis, and increased number of Howell-Jolly bodies, and numerous acanthocytes.

Another subchronic mouse inhalation (Hong *et al.*, 1989) study with female B6C3F1 mice using the same arsine levels for 12 weeks found similar hematological parameters affected, again accompanied by increased spleen weight, and with impaired compensatory erythropoiesis (bone marrow reduction of colony-forming unit erythroids/femur cells in culture) significant at the 2.5 ppm exposed group. Impaired erythropoiesis was observed in mice at both 0.5 ppm and 2.5 ppm arsine.

A subchronic study demonstrated altered immunocompetency of mice exposed to 2.5 ppm arsine for 14 days (Rosenthal *et al.*, 1989).

VI. Derivation of U.S. EPA Reference Concentration

Study	Blair *et al.*(1990a,b); Hong *et al.* (1989)
Study population	Fischer rats (15-16/ sex/group), B6C3F1 mice (15-16/sex/group) and Syrian Golden hamsters (15-16/sex/group)
Exposure method	Discontinuous whole-body inhalation exposures (0, 0.025, 0.5, 2.5, and 5.0 ppm)
Critical effects	Increased hemolysis, abnormal red blood cell morphology, and increased spleen weight
LOAEL	0.5 ppm
NOAEL	0.025 ppm
Exposure continuity	6 hr/day x 5 days/week
Exposure duration	14 consecutive days, 4 weeks, or 13 weeks
Average experimental exposure	0.004 ppm for NOAEL group
Human equivalent concentration	0.004 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factor	3 (lack of two-generation reproductive toxicity data)
Cumulative uncertainty factors	300
Inhalation reference exposure level	0.00001 ppm (0.01 ppb; 0.00005 mg/m^3; 0.05 $\mu g/m^3$)

Three comparative subchronic toxicity studies in B6C3F1 mice, Fischer 344 rats, and Syrian Golden hamsters (Blair *et al.*, 1990a,b; Hong *et al.*, 1989) reported similar hematological alterations at similar arsine concentrations. Additionally, splenomegaly and increased spleen weight were observed in all three species (Blair *et al.*, 1990a,b; Hong *et al.*, 1989). From these

studies a 0.025 ppm NOAEL and 0.5 ppm LOAEL were observed for increased hemolysis, altered RBC morphology, increased spleen weight, and impaired erythropoiesis.

Major strengths of the arsine RfC include the availability of a multiple species, multiple exposure study and the observation of a NOAEL and a dose-response relationship.

Major areas of uncertainty include the lack of adequate human health effects data and the lack of long-term exposure data.

VII. References

Blair P, Thompson M, Morrissey R, Moorman M, Sloane R, and Fowler B. 1990a. Comparative toxicity of arsine gas in B6C3f1 mice, Fischer 344 rats, and Syrian golden hamsters: System organ studies and comparison of clinical indices of exposure. Fund. Appl. Tox. 14:776-787.

Blair P, Thompson M, Bechtold M, Wilson R, Moorman M, and Fowler B. 1990b. Evidence for oxidative damage to red blood cells in mice induced by arsine gas. Toxicology 63:25-34.

HSDB. 1994. Hazardous Substance Data Bank. Micromedex Inc. Vol. 20.

Hong H, Fowler B, and Boorman G. 1989. Hematopoietic effects in mice exposed to arsine gas. Tox. Appl. Pharm. 97:173-182.

Rosenthal GJ, Fort MM, and Germolec DR. 1989. Effect of subchronic arsine inhalation on immune function and host resistance. Inh. Toxicol. 1:113-127.

U.S. EPA. 1994. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) Database. Reference concentration (RfC) for Arsine.

CHRONIC TOXICITY SUMMARY

BENZENE

(Benzol; Benzole; Cyclohexatriene)

CAS Registry Number: 71-43-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**60 μg/m^3**
Critical effect(s)	Lowered red and white blood cell counts in occupationally exposed humans
Hazard index target(s)	Circulatory system; teratogenicity; nervous system; immune system

II. Physical and Chemical Properties

(HSDB, 1994)

Molecular formula	C_6H_6
Molecular weight	78.1 g/mol
Description	Colorless liquid
Specific gravity	0.879 @ 25° C
Boiling point	80.1°C
Vapor pressure	100 mm Hg @ 26.1°C
Solubility	Soluble in ethanol, chloroform, ether, carbon disulfide, acetone, oils, and glacial acetic acid; slightly soluble in water
Conversion factor	1 ppm = 3.2 mg/m^3 @ 25° C

III. Major Uses or Sources

Benzene has been widely used as a multipurpose organic solvent. This use is now discouraged due to its high toxicity. Present uses include use as a raw material in the synthesis of styrene, phenol, cyclohexane, aniline, and alkyl benzenes in the manufacture of various plastics, resins, and detergents. Syntheses of many pesticides and pharmaceuticals also involve benzene as a chemical intermediate (HSDB, 1994). The tire industry and shoe factories use benzene extensively in their manufacturing processes. Annual demand in the U.S. was estimated to be 6 million tons in 1990 (HSDB, 1994). Benzene exposure also occurs as a result of gasoline and diesel exhaust combustion (Holmberg and Lundberg, 1985).

IV. Effects of Human Exposure

The primary toxicological effects of chronic benzene exposure are on the hematopoeitic system. Neurological and reproductive/developmental toxic effects are also of concern at slightly higher concentrations. Impairment of immune function and/or various anemias may result from the hematotoxicity. The hematologic lesions in the bone-marrow can lead to peripheral lymphocytopenia and/or pancytopenia following chronic exposure. Severe benzene exposures can also lead to life-threatening aplastic anemia. These lesions may lead to the development of leukemia years after apparent recovery from the hematologic damage (DeGowin, 1963).

Kipen *et al.* (1988) performed a retrospective longitudinal study on a cohort of 459 rubber workers, examining the correlation of average benzene exposure with total white blood cell counts taken from the workers. These researchers found a significant ($p < 0.016$) negative correlation between average benzene concentrations in the workplace and white blood cell counts in workers from the years 1940-1948. A reanalysis of these data by Cody *et al.* (1993) showed significant decreases in RBC and WBC counts among a group of 161 workers during the 1946-1949 period compared with their pre-exposure blood cell counts. The decline in blood counts was measured over the course of 12 months following start of exposure. During the course of employment, workers who had low monthly blood cell counts were transferred to other areas with lower benzene exposures, thus potentially creating a bias towards non-significance or removing sensitive subjects from the study population. Since there was a reported 75% rate of job change within the first year of employment, this bias could be highly significant. In addition, there was some indication of blood transfusions used to treat some "anemic" workers, which would cause serious problems in interpreting the RBC data, since RBCs have a long lifespan in the bloodstream. The exposure analysis in this study was performed by Crump and Allen (1984). The range of monthly median exposures was 30-54 ppm throughout the 12-month segment examined. Despite the above-mentioned potential biases, workers exposed above the median concentrations displayed significantly decreased WBC and RBC counts compared with workers exposed to the lower concentrations using a repeated measures analysis of variance.

Tsai *et al.* (1983) examined the mortality from all cancers and leukemia, in addition to hematologic parameters in male workers exposed to benzene for 1-21 years in a refinery from 1952-1978. The cohort of 454 included maintenance workers and utility men and laborers assigned to benzene units on a "regular basis". Exposures to benzene were determined using personal monitors; the median air concentration was 0.53 ppm in the work areas of greatest exposure to benzene. The average length of employment in the cohort was 7.4 years. The analysis of overall mortality in this population revealed no significant excesses. Mortality from all causes and from diseases of the circulatory system was significantly below expected values based on comparable groups of U.S. males. The authors concluded the presence of a healthy worker effect. An internal comparison group of 823 people, including 10% of the workers who were employed in the same plant in operations not related to benzene, showed relative risks for 0.90 and 1.31 for all causes and cancer at all sites, respectively ($p < 0.28$ and 0.23). A subset of 303 workers was followed for medical surveillance. Up to four hematological tests per year were conducted on these workers. Total and differential white blood cell counts, hemoglobin,

hematocrit, red blood cells, platelets and clotting times were found to be within normal (between 5% and 95% percentile) limits in this group.

An examination of 32 patients, who were chronically exposed to benzene vapors ranging from 150 to 650 ppm for 4 months to 15 years, showed that pancytopenia occurred in 28 cases. Bone marrow punctures revealed variable hematopoeitic lesions, ranging from acellularity to hypercellularity (Aksoy *et al.*, 1972).

Central nervous system disorders have been reported in individuals with pancytopenia following chronic occupational benzene exposure to unknown concentrations for an average length of time of 6 years (Baslo and Aksoy, 1982).

Runion and Scott (1985) estimated a composite geometric mean benzene concentration in various workplaces containing benzene to be 0.1 ppm (0.32 mg/m^3) (geometric standard deviation = 7.2 ppm, 23.3 mg/m^3). This estimate was based on samples collected by industrial hygienists between the years 1978 and 1983.

V. Effects of Animal Exposure

Mice have been shown to be more sensitive than rats or rabbits to the hematologic and leukemic effects of benzene (Sabourin *et al.*, 1989; IARC, 1982). Sabourin *et al.* (1988) showed that metabolism of benzene to the toxic hydroquinone, muconic acid, and hydroquinone glucuronide was much more prevalent in the mouse than in rats, whereas the detoxification pathways were approximately equivalent between the two species.

A study on the chronic hematological effects of benzene exposure in C57 Bl/6 male mice (5-6 per group) showed that peripheral lymphocytes, red blood cells and colony-forming units (CFUs) in the bone marrow and spleen were significantly decreased in number after treatment with 10 ppm (32.4 mg/m^3) benzene for 6 hours/day, 5 days/week for 178 days (Baarson *et al.*, 1984).

Male and female mice (9-10 per group) exposed to 100 ppm (324 mg/m^3) benzene or greater for 6 hours/day, 5 days/week for 2 weeks showed decreased bone marrow cellularity and a reduction of pluripotent stem cells in the bone marrow (Cronkite *et al.*, 1985). The decrease in marrow cellularity continued for up to 25 weeks following a 16-week exposure to 300 ppm (972 mg/m^3) benzene. Peripheral blood lymphocytes were dose-dependently decreased with benzene exposures of greater than 25 ppm (81 mg/m^3) for 16 weeks, but recovered to normal levels following a 16-week recovery period.

Hematologic effects, including leukopenia, were observed in rats exposed to mean concentrations of 44 ppm (143 mg/m^3) or greater for 5-8 weeks (Deichmann *et al.*, 1963). Exposure to 31 ppm (100 mg/m^3) benzene or less did not result in leukopenia after 3-4 months of exposure.

Inhalation of 0, 10, 31, 100, or 301 ppm (0, 32.4, 100.4, 324, or 975 mg/m^3) benzene for 6 hours/day, for 6 days resulted in a dose-dependent reduction in peripheral lymphocytes, and a

reduced proliferative response of B- and T-lymphocytes to mitogenic agents in mice (Rozen *et al.*, 1984). In this study, total peripheral lymphocyte numbers and B-lymphocyte proliferation to lipopolysaccharide were significantly reduced at a concentration of 10 ppm (32.4 mg/m^3). The proliferation of T-lymphocytes was significantly reduced at a concentration of 31 ppm (100.4 mg/m^3).

Aoyama (1986) showed that a 14-day exposure of mice to 50 ppm (162 mg/m^3) benzene resulted in a significantly reduced blood leukocyte count.

Reproductive and developmental effects have been reported following benzene exposure. Coate *et al.* (1984) exposed groups of 40 female rats to 0, 1, 10, 40, and 100 ppm (0, 3.24, 32.4, 129.6, or 324 mg/m^3) benzene for 6 hours/day during days 6-15 of gestation. In this study, teratologic evaluations and fetotoxic measurements were done on the fetuses. A significant decrease was noted in the body weights of fetuses from dams exposed to 100 ppm (324 mg/m^3). No effects were observed at a concentration of 40 ppm (129.6 mg/m^3).

Keller and Snyder (1986) reported that exposure of pregnant mice to concentrations as low as 5 ppm (16 mg/m^3) benzene on days 6-15 of gestation (6 hr/day) resulted in bone-marrow hematopoietic changes in the offspring that persisted into adulthood. However, the hematopoietic effects (e.g. bimodal changes in erythroid colony-forming cells) in the above study were of uncertain biological significance. In a similar later study, Keller and Snyder (1988) found that exposure of mice *in utero* to 20 ppm (64 mg/m^3) benzene on days 6-15 of gestation resulted in neonatal suppression of erythropoietic precursor cells and persistent, enhanced granulopoiesis. This effect was considered significant bone-marrow toxicity by the authors. No hematotoxicity was seen in this study at 10 ppm (32 mg/m^3).

An exposure of 500 ppm (1,600 mg/m^3) benzene through days 6-15 gestation was teratogenic in rats while 50 ppm (160 mg/m^3) resulted in reduced fetal weights on day 20 of gestation. No fetal effects were noted at an exposure of 10 ppm (Kuna and Kapp, 1981). An earlier study by Murray *et al.* (1979) showed that inhalation of 500 ppm benzene for 7 hours/day on days 6-15, and 6-18 of gestation in mice and rabbits, respectively, induced minor skeletal variations in the absence of maternal toxicity. Red and white blood cell counts in the adults of either species were measured by Murray *et al.* (1979) but were not significantly different from control animals. However, fetal mouse hematological effects were not measured.

Tatrai *et al.* (1980) demonstrated decreased fetal body weights and elevated liver weights in rats exposed throughout gestation to 150 mg/m^3 (47 ppm).

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Tsai *et al.* (1983)
Study population	303 Male refinery workers
Exposure method	Occupational exposures for 1-21 years
Critical effects	Hematological effects
LOAEL	Not observed
NOAEL	0.53 ppm
Exposure continuity	8 hr/day (10 m^3 per 20 m^3 day), 5 days/week
Exposure duration	7.4 years average (for the full cohort of 454) 32% of the workers were exposed for more than 10 years
Average occupational exposure	0.19 ppm
Human equivalent concentration	0.19 ppm
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10 (a healthy worker effect was observed)
Cumulative uncertainty factor	10
Inhalation reference exposure level	0.02 ppm (20 ppb; 0.06 mg/m^3; 60 $\mu g/m^3$)

Tsai *et al.* (1983) examined hematologic parameters in 303 male workers exposed to benzene for 1-21 years in a refinery from 1952-1978. Follow-up success was 99.3% in the entire cohort of 359. A total of approximately 1400 samples for hematological tests and 900 for blood chemistry tests were taken between 1959 and 1979. Exposures to benzene were determined using personal monitors. Data consisting of 1394 personal samples indicated that 84% of all benzene samples were less than 1 ppm; the median air concentration of benzene was 0.53 ppm in the work areas of greatest exposure to benzene ("benzene related areas", for example, production of benzene and cyclohexane and also of cumene). The average length of employment in the cohort was 7.4 years. Mortality from all causes and from diseases of the circulatory system was significantly below expected values based on comparable groups of U.S. males. The authors concluded the presence of a healthy worker effect. An analysis using an internal comparison group of 823 people, including 10% of the workers who were employed in the same plant in operations not related to benzene, showed relative risks for 0.90 and 1.31 for all causes and cancer at all sites, respectively ($p < 0.28$ and 0.23). Total and differential white blood cell counts, hemoglobin, hematocrit, red blood cells, platelets and clotting times were found to be within normal (between 5% and 95% percentile) limits in this group.

Although the study by Tsai *et al.* (1983) is a free-standing NOAEL, the endpoint examined is a known sensitive measure of benzene toxicity in humans. In addition, the LOAEL for the same endpoint in workers reported by Cody *et al.* (1993) help form a dose-response relationship and also yeild an REL which is consistent with that derived from Tsai *et al.* (1983). The study by Cody *et al.* (1993), since it failed to identify a NOAEL and was only for a period of 1 year, contained a greater degree of uncertainty in extrapolation to a chronic community Reference

Exposure Level. Therefore the study by Tsai *et al.* (1983) was used as the basis for the chronic REL for benzene.

In the Cody *et al.* (1993) study, significant hematological effects, including reduced RBC and WBC counts, were observed in 161 male rubber workers exposed to median peak concentrations (i.e. only the peak concentrations for any given exposure time were reported) of 30-54 ppm or more for a 12-month period during 1948. The 30 ppm value was considered a 1-year LOAEL for hematological effects. In this rubber plant, workers who had blood dyscrasias were excluded from working in the high benzene units. Furthermore, individual workers having more than a 25% decrease in WBC counts from their pre-employment background count were removed from the high benzene units and placed in other units with lower benzene concentrations. Sensitive individuals therefore could have been excluded from the analysis. The 30 ppm value is the low end of the range of median values (30-54 ppm) reported by Crump and used in the Kipen *et al.* (1988) and Cody *et al.* (1993) studies. An equivalent continuous exposure of 10.7 ppm can be calculated by assuming 10 m^3 per 20 m^3 day, and adjusting for a normal 5 day work week. Application of uncertainty factors for subchronic exposures, estimation of a NOAEL, and protection of sensitive subpopulations, results in an REL of 0.01 ppm.

Ward et al. (1996) determined a relationship between occupational exposures to benzene and decreased red and white cell counts, with a modeled dose-response relationship that indicated a possibility for hematologic effects at concentrations below 5 ppm. However, no specific measures of the actual effects at these concentrations below 2 ppm were taken, and the Tsai et al. (1983) data was not considered in their analysis. The purpose of this study was to characterize the trend for effects at low concentrations of benzene. A NOAEL or LOAEL was not identified in the study. The selection of a NOAEL of 0.53 ppm is therefore not inconsistent with the results of the Ward et al. (1996) study.

The human data presented by Tsai and associates was selected over animal studies because the collective human data was considered adequate in terms of sample size, exposure duration, and health effects evaluation. For comparison, the chronic inhalation study in mice by Baarson *et al.* (1984) showed that bone-marrow progenitor cells were markedly suppressed after intermittent exposures (6 hr/day, 5 days/week) to 10 ppm benzene. An extrapolation of this value to an equivalent continuous exposure resulted in a concentration of 1.8 ppm. Application of uncertainty factors of 10 each for inter- and intraspecies variability, and estimation of a NOAEL from the LOAEL would result in an REL of 2 ppb.

VII. References

Aksoy M, Dincol K, Erdem S, Akgun T, and Dincol G. 1972. Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. Br. J. Ind. Med. 29:56-64.

Aoyama K. 1986. Effects of benzene inhalation on lymphocyte subpopulations and immune response in mice. Toxicol. Appl. Pharmacol. 85:92-101.

Baarson KA, Snyder CA, and Albert RE. 1984. Repeated exposure of C57Bl/6 mice to inhaled benzene at 10 ppm markedly depressed erythrocyte colony formation. Toxicology Lett. 20:337-342.

Baslo A, and Aksoy M. 1982. Neurological abnormalities in chronic benzene poisoning. A study of six patients with aplastic anemia and two with preleukemia. Environ. Res. 27:457-465.

Coate WB, Hoberman AM, and Durloo RS. 1984. Inhalation teratology study of benzene in rats. in, Advances in Modern Environmental Toxicology, Vol. VI. Applied Toxicology of Petroleum Hydrocarbons. Princeton Scientific Publishers, Inc. Princeton, NJ.

Cody RR, Strawderman WW, and Kipen HM. 1993. Hematologic effects of benzene. J. Occup. Med. 35(8):776-782.

Cronkite EP, Drew RT, Inoue T, and Bullis JE. 1985. Benzene hematotoxicity and leukemogenesis. Am. J. Ind. Med. 7:447-456.

Crump K, and Allen B. 1984. Quantitative estimates of risk of leukemia from occupational exposure to benzene. Occupational Safety and Health Administration; Docket H-059B. As cited in: Cody, R.R., Strawderman, W.W., and Kipen, H.M. 1993. Hematologic effects of benzene. J. Occup. Med. 35(8):776-782.

DeGowin RL. 1963. Benzene exposure and aplastic anemia followed by leukemia 15 years later. J. Am. Med. Assoc. 185(10):748-751.

Deichmann WB, MacDonald WE, and Bernal E. 1963. The hematopoietic tissue toxicity of benzene vapors. Toxicol. Appl. Pharmacol. 5:201-224.

HSDB. 1994. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM version). Micromedex, Inc., Denver, CO (edition expires 11/31/94).

Holmberg B, and Lundberg P. 1985. Benzene: Standards, occurrence, and exposure. Am. J. Ind. Med. 7:375-383.

IARC. 1982. International Agency for Research on Cancer. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Industrial Chemicals and Dyestuffs. Volume 29. pg. 95-148.

Keller KA, and Snyder CA. 1986. Mice exposed in utero to low concentrations of benzene exhibit enduring changes in their colony forming hematopoietic cells. Toxicology 42:171-181.

Keller KA, and Snyder CA. 1988. Mice exposed in utero to 20 ppm benzene exhibit altered numbers of recognizable hematopoietic cells up to seven weeks after exposure. Fundam. Appl. Toxicol. 10:224-232.

Kipen HM, Cody RP, Crump KS, Allen BC, and Goldstein BD. 1988. Hematologic effects of benzene: A thirty-five year longitudinal study of rubber workers. Toxicol. Ind. Health. 4(4):411-430.

Kuna RA, and Kapp RW. 1981. The embryotoxic/teratogenic potential of benzene vapor in rats. Toxicol. Appl. Pharmacol. 57:1-7.

Murray FJ, John JA, Rampy LW, Kuna RA, and Schwetz BA. 1979. Embryotoxicity of inhaled benzene in mice and rabbits. Am. Ind. Hyg. Assoc. J. 40:993-998.

Rozen MG, Snyder CA, and Albert RE. 1984. Depressions in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. Toxicol. Lett. 20:343-349.

Runion HE, and Scott LM. 1985. Benzene exposure in the United States 1978-1983: An overview. Am. J. Ind. Med. 7:385-393.

Sabourin PJ, Bechtold WE, Birnbaum LS, Lucier G, and Henderson RF. 1988. Differences in the metabolism and disposition of inhaled [3H]benzene by F344/N rats and B6C3F1 mice. Toxicol. Appl. Pharmacol. 94:128-140.

Sabourin PJ, Bechtold WE, Griffith WC, Birnbaum LS, Lucier G, and Henderson RF. 1989. Effect of exposure concentration, exposure rate, and route of administration on metabolism of benzene by F344 rats and B6C3F1 mice. Toxicol. Appl. Pharmacol. 99:421-444.

Tatrai E, Ungvary GY, Hudak A, Rodics K, Lorincz M, and Barcza GY. 1980. Concentration dependence of the embryotoxic effects of benzene inhalation in CFY rats. J. Hyg. Epidem. Micro. Immunol. 24(3):363-371.

Tsai SP, Wen CP, Weiss NS, Wong O, McClellan WA, and Gibson RL. 1983. Retrospective mortality and medical surveillance studies of workers in benzene areas of refineries. J. Occup. Med. 25(9):685-692.

Ward E, Hornburg R, Morris J, Rinsky R, Wild D, Halperin W, and Guthrie W. 1996. Risk of low red or white blood cell count related to estimated benzene exposure in a rubberworker cohort (1940-1975). Am. J. Ind. Med. 29:247-257.

CHRONIC TOXICITY SUMMARY

BENZIDINE

(4,4'-bianiline; 4,4'-biphenyldiamine; 4,4'-diaminobiphenyl; 4,4'-diphenylenediamine; (1,1'-biphenyl)-4,4'-diamine; C.I. Azoic Diazo Component 112)

CAS Registry Number: 92-87-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**10 µg/m^3**
Oral reference exposure level	**3 µg/kg-day**
Critical effect(s)	Vacuolization of the brain and cytological effect on the liver of mice
Hazard index target(s)	Nervous system; alimentary system

II. Chemical Property Summary (from HSDB, 1995, except as noted)

Molecular formula	$C_{12}H_{12}N_2$
Molecular weight	184.2 g/mol
Description	Grayish-yellow, white or reddish-gray solid
Vapor pressure	0.0005 mm Hg @ 25°C (Mabey *et al.*, 1982)
Solubility	0.4g/l cold water; 9.34g/l boiling water; 200g/l boiling alcohol; 20g/l ether; 0.4g/l water @ 12°C
Conversion factor	7.53 µg/m^3 = 1 ppb at 25ºC

III. Major Uses and Sources

Benzidine has been used primarily in the synthesis of dyes for use on cloth, paper and leather. Benzidine has not been available commercially since 1974, although small sources arise from imports, laboratory use, and limited dye synthesis (HSDB, 1995). Many benzidine-based dyes may also produce benzidine in the body by metabolic processes (Boeniger, 1978).

IV. Effects of Human Exposure

Sensitization to benzidine among patients reporting occupational allergy was described (Grimalt and Romaguera, 1981). Among 4600 patients tested, 231 (~5%) showed sensitivity to benzidine dihydrochloride, benzidine chlorohydrate, or pure benzidine (all 3% in petrolatum). Biopsy examination of the skin made after removal of the skin test patch showed epidermal and dermal involvement characterized by exocytosis, spongiosis, and microvesiculation within the first 18

hours, which proceeded to pericapillar disposition and swelling of the endothelial cells by 24 hours. After 2 days, the dermal reaction progressed with increasingly intense infiltration (of both lymphocytes and neutrophils) and necrotic epidermal lesions. At one week, the epidermis became acanthotic. The time to resolution was not discussed. The exposure level and duration which led to the sensitization was not characterized.

The activity of natural killer cells in dyestuff workers exposed to aromatic amines (benzidine and β-naphthylamine) was examined (Tanigawa *et al.*, 1990). Although there was no difference in gross natural killer cell activity between exposed workers and the control group, the unit natural killer cell activity (cytotoxic potential per natural killer cell) was reduced in exposed workers relative to the control group. Exposure levels and duration of exposure were not reported in the study.

No statistically significant increases in birth defects among people in the vicinity of the Drake Superfund site in Clinton County, Pennsylvania were reported (Budnick *et al.*, 1984). The site is known to be contaminated with β-naphthylamine, benzidine, and benzene.

V. Effects of Animal Exposure

The toxicity of benzidine dihydrochloride to mice chronically exposed to the compound in drinking water was reported (Littlefield *et al.*, 1983). Two populations of mice were examined, one the result of a cross between BALB/cStCr1C3Hf/Nctr males with C57BL/6JfC3Hf/Nctr females termed F_1, and the other a monohybrid cross of the F_1 mice termed MC. The study consisted of treating 72 mice/sex/dose with benzidine dihydrochloride for 33 months in drinking water at concentrations of 0, 30, 40, 60, 80, 120, and 160 ppm (males) and 0, 20, 30, 40, 60, 80, and 120 ppm (females) with the exception of the two lowest dose groups which consisted of 120 and 96 mice/sex/group. Among the treated animals, mortality was increased primarily from deaths due to tumors and a treatment-related decrease in body weight gain was observed during the course of the study.

Non-cancer adverse health effects were observed among the treated animals. Significant effects observed among the lowest dose groups (20 ppm for females and 30 ppm for males) included brain vacuolization which was characterized as bilaterally symmetric foci of spongy change in the internal capsule of the cerebrum (observed in both male and female mice), and cytological alteration of the liver (females only). Other adverse effects which were found to be dose-related include spleen pigmentation in male mice at 120 ppm and bile duct hyperplasia in females at 60 ppm and males at 160 ppm.

Monohybrid cross and F_1 cross mice (as described by Littlefield *et al.*,1983) were treated with benzidine dihydrochloride in drinking water (Nelson *et al.*, 1982). Animals (24-72 mice/sex/cross/dose group) were treated for 40, 60, or 80 weeks with 0, 30, 60, 120, 200, 400 ppm benzidine dihydrochloride. Although the focus of the study was carcinogenesis, some non-cancer endpoints were reported. All animal groups showed weight reduction at some dose level, with monohybrid male mice the most sensitive, showing a 10% reduction in weight relative to

controls within 16 weeks at the lowest dose level (30 ppm). Water consumption decreased in animals in dose groups above 30 ppm, although the magnitude of this effect was not as pronounced in the animals treated for 80 weeks, suggesting some acclimatization. Hemosiderosis of the spleen was termed significant in the groups of the 40- and 60-week studies and at 80 weeks among males only (pooled numbers from treated animals and controls groups were analyzed to provide indicators of possible effects, thus dose group was not reported for this and the following effects). Hyperplasia of the bile duct and erythropoiesis in the spleen were significant effects in female mice in the 60- and 80-week studies.

A range-finding study was conducted for chronic carcinogenicity studies, exposing C57BL × C3H F_1 mice (numbers unspecified) to 0.01% or 0.08% benzidine dihydrochloride in feed for 6 weeks (Rao *et al.*, 1971). Observed effects included decreased body weight gain, decreased liver and kidney weights, cloudy swelling of the liver, degeneration of the kidney tubules, and hyperplastic change of the myeloid cells of the bone marrow, and the lymphoid cells of the spleen and thymic cortex.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Littlefield *et al.*, 1983
Study population	Mice
Exposure method	Oral (drinking water)
Critical effects	Vacuolization of the brain; cytological alterations of the liver; spleen pigmentation
LOAEL	20 ppm in drinking water (females)
NOAEL	Not observed
Exposure continuity	Not applicable
Average experimental exposure	2.7 mg/kg-day
Exposure duration	33 months
Subchronic uncertainty factor	1
LOAEL uncertainty factor	10
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	1,000
Oral reference exposure level	0.003 mg/kg-day (US EPA RfD)
Inhalation extrapolation factor	3,500 $\mu g/m^3$ per mg/kg-day
Inhalation reference exposure level	10 $\mu g/m^3$

In the absence of data demonstrating toxicity to humans and animals from exposure to benzidine by the inhalation route, an oral toxicity study has been selected for the development of the chronic reference exposure level. The Littlefield *et al.* (1983) study represents the most thorough reporting of the non-cancer adverse effects of long-term benzidine administration. The US EPA based its reference dose (RfD) on the Littlefield *et al.* (1983) study showing toxicity to the brain and liver of mice exposed to benzidine in drinking water (US EPA, 1995). The evidence of toxicity is consistent among the studies in which benzidine is administered by the oral route, with

the common reporting of hematosiderosis or pigmentation of the spleen and hyperplasia of the bile duct. Toxic effects showed dose-response relationships in animals. The most sensitive site of benzidine's toxicity identified in this study is the vacuolization of brain regions and cytological alterations of the liver which were observed in the lowest treatment group (20 ppm in female mice). This level of exposure was taken to be the LOAEL. The US EPA, in its calculation of the RfD, used the authors' reported level of animal drinking water consumption and body weight and the molecular weight ratio of the free base (184.23) to that of the dihydrochloride (257.16) to calculate an average daily dosing rate of 2.7 mg/kg-day.

The major strength of the REL is the use of lifetime exposure data. The major uncertainties are the lack of adequate human data, the lack of a NOAEL observation, and the lack of comprehensive, long-term multiple-dose multiple-species inhalation studies.

VII. References

Boeniger M. 1978. An investigation of the metabolic reduction of benzidine azo dyes to benzidine and its metabolites, and their possible relationship to carcinoma of the bladder in man. Cincinnati, OH: US Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health (unpublished).

Budnick LD, Sokal DC, Falk H, Logue JN, and Fox JM. 1984. Cancer and birth defects near the Drake Superfund site, Pennsylvania. Arch Environ Health, 39:409-13.
Grimalt, F. and Romaguera, C. 1981. Cutaneous sensitivity to benzidine. Dermatosen, 29:95-7.

HSDB. 1996. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex, Inc., Denver, Colorado (Edition expires 7/31/96).

Littlefield NA, Nelson CJ, and Frith CH. 1983. Benzidine dihydrochloride: toxicological assessment in mice during chronic exposures. J Toxicol Environ Health, 12:671-85.

Mabey WR, Smith JH, Podoll RT *et al.* 1982. Aquatic fate process data for organic priority pollutants. US Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC. EPA-440/4-81-041.

Nelson CJ, Baetcke KP, Frith CH, Kodell RL, and Schieferstein G. 1982. The influence of sex, dose, time, and cross on neoplasia in mice given benzidine dihydrochloride. Toxicol Appl Pharmacol, 64:171-86.

Rao KVN, Rust JH, Mihailovich N, Vesselinovitch SD, and Rice JM. 1971. Subacute toxicity of benzidine in the young adult mice. Federation Proceedings, 30:444.

Tanigawa T, Araki S, Ishizu S, Morita T, Okazaki H, and Minato N. 1990. Natural killer cell activity in workers exposed to benzidine and beta-naphthylamine. Br J Ind Med, 47:338-41.

U.S. EPA. 1995. United States Environmental Protection Agency. Documentation of the reference dose for chronic oral exposure (RfD) for benzidine. Integrated Risk Information System (IRIS on-line). US EPA: Washington, DC.

CHRONIC TOXICITY SUMMARY

BERYLLIUM and BERYLLIUM COMPOUNDS

(beryllium-9; glucinium; glucinum; beryllium metallic)
CAS Registry Number: 7440-41-7

(Beryllium oxide; beryllia; beryllium monoxide)
CAS Registry Number: 1304-56-9

(Beryllium hydroxide; beryllium hydrate; beryllium dihydroxide)
CAS Registry Number: 13327-32-7

(Beryllium sulfate; sulfuric acid, beryllium salt)
CAS Registry Number: 13510-49-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.001 μg Be/m^3**
Critical effect(s)	Berylliosis in non-occupationally exposed humans
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties Summary (ATSDR, 1993)

Molecular formula	Be	BeO	$Be(OH)_2$	$BeSO_4$
Molecular weight	9.012	25.01	43.03	105.07
Description	Solid gray, hexagonal structure	White light, amorphous powder	White amorphous powder or crystalline	Colorless tetragonal crystals
Vapor pressure	10 mm Hg at 1860 ^oC	No data	No data	No data
Solubility	Insoluble in water; metal soluble in dilute acid and alkali, oxide and hydroxide species soluble in concentrated acid and alkali			
Conversion factor	Not applicable			

III. Major Uses and Sources

Beryllium is a metallic element mined from bertrandite and beryl mineral ores. As the lightest structural metal, beryllium is used in the space, aircraft and nuclear industries in a variety of components including air craft disc brakes, x-ray transmission windows, vehicle optics, nuclear reactor neutron reflectors, fuel containers, precision instruments, rocket propellants, navigational

systems, heat shields, and mirrors. In addition to the 4 species listed, there are at least 13 other beryllium containing compounds including other salts, ores and alloys.

Beryllium alloys, especially the hardest alloy beryllium copper, are used in electrical equipment, precision instruments, springs, valves, non-sparking tools, and in molds for injection-molded plastics for automotive, industrial and consumer applications. Beryllium oxide is used in high-technology ceramics, electronic heat sinks, electrical insulators, crucibles, thermocouple tubing, and laser structural components. Other beryllium compounds, containing chloride, nitrate, fluoride and sulfate, are utilized as chemical reagents or generated from the refining of beryllium-containing ores.

Beryllium is naturally emitted into the atmosphere by windblown dusts and volcanic particles. However, the major emission source is the combustion of coal and fuel oil, which releases beryllium-containing particulates and ash. Other beryllium-releasing industrial procedures include ore processing, metal fabrication, beryllium oxide production, and municipal waste production (ATSDR, 1993). Beryllium also occurs in tobacco smoke.

IV. Effects of Human Exposure

The respiratory tract is the major target organ system in humans following the inhalation of beryllium. The common symptoms of chronic beryllium disease include shortness of breath upon exertion, weight loss, cough, fatigue, chest pain, anorexia, and overall weakness. Most studies reporting adverse respiratory effects in humans involve the occupational exposure to beryllium. Exposure to soluble beryllium compounds is associated with acute beryllium pneumonitis (Eisenbud *et al.*, 1948) while exposure to both soluble or insoluble beryllium compounds may result in obstructive and restrictive diseases of the lung, called chronic beryllium disease (berylliosis) (Cotes *et al.*, 1983; Johnson, 1983; Infante *et al.*, 1980; Kriebel *et al.*, 1988a; Metzner and Lieben, 1961). Overall, the total number of beryllium-related disease cases has declined since the adoption of industrial standards (Eisenbud and Lisson, 1983; ATSDR, 1993).

Historically, beryllium pneumonitis has been associated with occupational concentrations over 0.1 mg Be/m^3, primarily as beryllium sulfate or beryllium fluoride (Eisenbud *et al.* 1948). But the atmospheric concentrations related to chronic beryllium disease have been more difficult to define, in part due to the lack of individual exposure estimates, especially in the studies derived from the berylliosis case registries (Infante *et al.*, 1980; Lieben and Metzner, 1959). However, Infante and associates (1980) reported significant increased mortality due to non-neoplastic respiratory disease in beryllium-exposed workers, and noted one case of chronic berylliosis in a worker following 7 years exposure to $\leq$ 2 µg Be/m^3. In a 30-year follow-up study of 146 beryllium-exposed workers, Cotes *et al.* (1983) identified seven cases of chronic beryllium related disease (146 workers examined). All the cases were exposed to beryllium oxide or hydroxide, but in a wide range of retrospectively estimated doses (over 3000 samples from 1952 to 1960). The estimated average daily exposure did not exceed 2 µg/m^3 for the ten site/process classifications, but 318 samples did exceed 2 µg Be/m^3 (20 samples greater than 25 µg Be/m^3).

No atmospheric samples were available after 1963, even though the exposure occurred through 1973. The LOAEL for occupationally-induced berylliosis observed in this study was estimated from uncertain exposure data to be less than 2 µg Be/m^3.

One cross-sectional study (Kriebel *et al.*, 1988a; Kriebel *et al.*, 1988b) estimated beryllium exposure levels for 309 workers originally surveyed in 1977, with a median duration of exposure of 17 years (range 2 to 39 years). Historic plant beryllium levels were estimated as high as 100 µg Be/m^3, and, even as late as 1975, some job classifications exceeded 10 µg Be/m^3. The workers' median cumulative exposure was 65 µg Be/m^3-years (range 0.1 to 4400 µg Be/m^3-years) with a mean lifetime exposure median estimate of 4.3 µg/m^3 (range 0.01 to 150 µg/m^3). Spirometric measurement of pulmonary function, chest x-rays, and arterial blood gas measurements were collected. Decrements in lung function, as defined by forced vital capacity (FVC) and forced expiratory volume (FEV_1), were associated with cumulative exposure up to 20 years prior to the health survey, even in workers with no radiographic abnormalities. Differences in alveolar-arterial oxygen gradient were associated with cumulative exposure in the 10 years prior to the study. These endpoints give a LOAEL of 39 µg/m^3-years (geometric mean cumulative exposure) for decrements in pulmonary function and changes in arterial blood gases.

Non-occupational beryllium-related chronic disease has been reported in individuals residing in the vicinity of beryllium manufacturing industries (Eisenbud *et al.*, 1949; Metzner and Lieben, 1961). An early cross-sectional study (Eisenbud *et al.*, 1949) described 11 cases of non-occupational berylliosis after examining (x-ray and clinical) approximately 10,000 residents near a beryllium fabrication facility. Ten of the cases resided within 3/4 mile of the plant (up to 7 years duration), and five cases resided within 1/4 mile. The authors approximated a 1% disease incidence within 1/4 mile (500 individuals). Atmospheric sampling in 1947 identified an average beryllium level of 0.2 µg Be/m^3 at 1/4 mile decreasing to 0 µg Be/m^3 at 10 miles, but samples varied widely (up to 100 fold) over the 10 week sampling period. Utilizing current and historic exposure estimates based on discharge, process, inventory and building design changes, this study estimated a chronic LOAEL in the range of 0.01 to 0.1 µg Be/m^3 for continuous exposure to beryllium compounds, based on the development of chronic berylliosis.

Metzner and Lieben (1961) also reported 26 cases of chronic berylliosis in a approximate population of 100,000 living within 7 miles of a refining and alloy fabrication plant (duration 6 to 19 years). Neighborhood exposure assessment conducted over 14 months (1958-59) identified a mean level of 0.0155 µg Be/m^3, with 10% of the samples registering over 0.03 µg Be/m^3. Limited measurements conducted earlier at the site were higher (1.0 to 1.8 µg Be/m^3 in 1953 and 0.91 to 1.4 µg Be/m^3 in 1954).

Chronic beryllium disease appears to involve a cell-mediated immune response (especially granulomatous reactions found in the lungs of sensitive individuals). Humans exposed to beryllium compounds have demonstrated increased T-cell activity (*in vitro*) and histological abnormalities of the lymph nodes (Cullen *et al.*, 1987; Johnson, 1983). Johnson (1983) described granuloma of lymph nodes and chronic interstitial pneumonitis in a small number of beryllium metal handling machinists (LOAEL 4.6 µg Be/m^3). A second study identified granulomatous lung lesions, scarred lung tissue, and breathing difficulties in workers from a

precious metal refining facility exposed to a mixture of beryllium and other metals (Cullen *et al.*, 1987). Also, altered proliferative responses of lymphocytes obtained by bronchoalveolar lavage indicated increased T-cell activity in vitro. This study reported a LOAEL of 1.2 µg Be/m^3 for the immunological and respiratory endpoints.

V. Effects of Animal Exposure

Three chronic studies, two in rats (Vorwald and Reeves, 1959; Reeves *et al.*, 1967) and one in guinea pigs (Reeves *et al.*, 1970), observed adverse inflammatory and proliferative respiratory changes following inhalation exposure to beryllium compounds. Vorwald and Reeves (1959) observed inflamed lungs and fibrosis in rats exposed to 0.006 mg Be/m^3 (as BeO) for an unspecified duration. A later study exposed Sprague-Dawley CD rats for 72 weeks (7 hr/d, 5 d/wk) to 34.25 µg Be/m^3 from $BeSO_4$ (Reeves *et al.*, 1967) . Gross and histological changes observed in exposed versus unexposed rats included increased lung weight, inflamed lungs, emphysema, arteriolar wall thickening, granulomas, fibrosis, and proliferative responses within the alveoli (LOAEL 34.25 µg Be/m^3). In guinea pigs exposed to either 0, 3.7, 15.4 or 29.3 µg Be/m^3 (from the sulfate) for 6 hours/day, 5 days/week for up to 1 year, respiratory alterations observed in the beryllium-exposed groups included increased tracheobronchial lymph node and lung wet weights, interstitial pneumonitis and granulomatous lesions. These adverse respiratory effects were observed in all the beryllium dosed groups, suggesting a chronic inhalation LOAEL of 3.7 µg Be/m^3.

Wagner *et al.* (1969) exposed monkeys, rats and hamsters to 0.21 and 0.62 mg Be/m^3 as fumes from bertrandite or beryl ore, respectively. Rats displayed the more severe effects including bronchial lymphocytic infiltrates, abscesses, consolidated lobes, and granulomatous lesions after exposure to 0.21 mg Be/m^3 from bertrandite ore, and inflamed lungs, fibrosis and granuloma after exposure to 0.62 mg Be/m^3 from beryl ore 6 hours/day, 5 days/week for up to 17 months. Lung inflammation was observed in the exposed monkeys, and a few granulomatous lung lesions were observed in the hamsters after similar exposure conditions (up to 23 months).

Immunological effects have been observed in a few subchronic studies (Schepers, 1964; Schepers *et al.*, 1957; Stiefel *et al.*, 1980). Schepers (1964) exposed monkeys (*Macacus mullata*) to three soluble forms of beryllium (BeF_2, $BeSO_4$, $BeHPO_4$) daily for 6 hours/day over 7 to 30 days. Increased lung weight, inflammation, emphysema and fibrosis of the lung were observed after 17 days at 0.198 mg Be/m^3 (as $BeSO_4$). Histological examination found pleuritis, congestion, emphysema, consolidation and edema of the lung. Immunological effects were seen as hyperplasia of the lymph nodes typical of immune activation after 7 to 18 days exposure to either 0.198 or 0.184 mg Be/m^3 as the sulfate or fluoride. A subchronic inhalation study reported immunological effects as increased beryllium-specific stimulation of T-lymphocytes *in vitro* from Wistar rats and guinea pigs exposed daily (6 hours/day) over 10 weeks (LOAEL 0.5 mg/m^3) (Stiefel *et al.*, 1980). However, a subchronic inhalation study on Wistar and Sherman rats (Schepers *et al.*, 1957), which did observe multiple lung alterations including granulomas (LOAEL 35 µg Be/m^3), did not find any accompanying immunological effects after 30 days discontinuous exposure (5-6 d/wk, 4-8 hr/d) to beryllium fumes from $BeSO_4$.

VI. Derivation of Chronic Reference Exposure Levels

Derivation of Inhalation Reference Exposure Level

Study	Eisenbud *et al.* (1949)
Study population	Approximately 10,000 individuals within 2 miles of beryllium manufacturing plant
Exposure method	Environmental exposure
Critical effects	Pulmonary berylliosis
LOAEL	0.03 µg/m^3 (geometric mean of range of measured exposures associated with berylliosis of 0.01 to 0.1 µg/m^3)
NOAEL	Not observed
Exposure continuity	Continuous
Average exposure	Estimated to be approximately 0.3 µg/m^3 (historical exposures estimated to be 10-fold higher than measured values) for LOAEL group
Human equivalent concentration	0.3 µg/m^3 for LOAEL group
Exposure duration	Up to 7 years
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.001 µg/m^3

Eisenbud *et al.* (1949) reported 11 cases of nonoccupational chronic pulmonary granulomatosis (berylliosis) found among approximately 10,000 residents screened (health survey and x-ray) in the vicinity of a beryllium manufacturing plant. Ten of the cases resided within 3/4 mile of the facility, but neither these individuals nor their spouses had occupational contact with the plant. Five cases occurred within 1/4 mile of the plant from an approximate population of 500 residents. Though this study gave minimal case and population descriptions, environmental exposure assessment was conducted (fixed and mobile stations over 10 weeks) at the time of study (1947), and historical estimates were developed from earlier measurements, downwind effluent models, inventory, process and building design changes over time (plant operated 1935 to 1947). In 1947, air concentrations ranged from 2 µg Be/m^3 at 1/4 mile to 0 µg Be/m^3 at 2 miles distance (detection limit 0.001 µg Be/m^3). The estimated concentration at 3/4 mile distance from the plant was 0.01 µg Be/m^3. The authors estimated the airborne beryllium concentration associated with berylliosis as 0.01 to 0.10 µg Be/m^3. This estimate included multiplication of the 0.01 µg/m^3 concentration by a factor of 10 to account for greater historical exposures.

One other report describes 26 cases of berylliosis due to environmental exposure to beryllium plant effluent (Metzner and Lieben, 1961). This study reported a similar mean air concentration of 0.0155 µg Be/m^3, while limited historic measurements ranged from 0.91 to 1.8 µg Be/m^3.

Occupational studies have reported berylliosis and/or alterations in pulmonary function after exposure to higher concentrations of beryllium (2- to 10-fold). Cotes *et al.* (1983) reported on 146 workers surveyed three times since 1963 (1963, 1973, and 1977). Exposure assessment was based on plant sampling from 1952 to 1963. The estimated overall daily average was < 2 µg Be/m^3, however, a wide range of individual integrated exposures was estimated. Seven cases of berylliosis-related disease were observed in 130 workers examined in 1973. No association was seen between lung function and estimated exposure in normal subjects. However, Kriebel *et al.* (1988a; 1988b) did find decrements in lung function significantly associated with cumulative exposure to beryllium. Lifetime beryllium exposure histories were estimated for 309 of 350 workers (mean duration 17 years) and 297 underwent medical testing. The median cumulative exposure was 65 µg Be/m^3-years (mean cumulative 37 µg Be/m^3) and the mean lifetime exposure 3 µg Be/m^3. After controlling for age, height, and smoking in multivariate regression models, decrements in lung function (FVC and FEV_1) were associated with cumulative exposure to beryllium in the period up until 20 years before the survey.

The major strength of the REL is the use of human data among residentially-exposed persons. The major uncertainties are the lack of a NOAEL observation, the lack of long-term exposure data and the difficulty of estimating exposures, and the lack of chronic exposure data.

Derivation of Chronic Oral Reference Exposure Level

Study	Schroeder and Mitchner, 1975
Study population	Rats
Exposure method	Drinking water
Critical effects	No adverse effects at dose given
LOAEL	Not observed
NOAEL	5 ppm in water (0.54 mg/kg bw-day)
Exposure continuity	Continuous
Exposure duration	Lifetime
Average experimental exposure	0.54 mg/kg bw-day
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies factor	10
Cumulative uncertainty factor	100
Oral reference exposure level	0.005 mg/kg bw-day

*Conversion Factors: 5 ppm (5 mg/L) x 0.035 L/day / 0.325 kg bw = 0.54 mg/kg bw/day

The Oral Reference Exposure Level (REL) for beryllium is the U.S. EPA's Reference Dose for chronic oral exposure (RfD) (IRIS, 1996). Fifty-two weanling Long-Evans rats of each sex received 0 or 5 ppm beryllium (as $BeSO_4$, beryllium sulfate) in drinking water (Schroeder and Mitchner, 1975). Exposure was for the lifetime of the animals. At natural death the rats were

dissected and gross and microscopic changes were noted in heart, kidney, liver, and spleen. There were no effects of treatment on these organs or on lifespan, urinalysis, serum glucose, cholesterol, and uric acid, or on numbers of tumors. Male rats experienced decreased growth rates from 2 to 6 months of age. Similar studies were carried out on Swiss (CD strain) mice in groups of 54/sex at doses of approximately 0.95 mg/kg/day (Schroeder and Mitchner, 1975). Female animals showed decreased body weight compared with untreated mice at 6 of 8 intervals. Male mice exhibited slight increases in body weight. These effects were not considered adverse, therefore, 0.95 mg/kg/day is considered a NOAEL. An unpublished investigation by Cox *et al.* (1975) indicates a much higher dose level (approximately 25 mg/kg/day) in the diet may be a NOEL.

The uncertainty factor (UF) of 100 reflects a factor of 10 each for interspecies conversion and for the protection of sensitive human subpopulations. No modifying factor (MF) was used.

This RfD is limited to soluble beryllium salts. Data on the teratogenicity or reproductive effects of beryllium are limited. It has been reported to produce embryolethality and terata in chick embryos.

U.S. EPA stated its confidence in the RfD as: Study - Low; Data Base - Low; and RfD - Low. Confidence in the study is rated as low because only one dose level was administered. Although numerous inhalation investigations and a supporting chronic oral bioassay in mice exist, along with the work by Cox *et al.* (1975) which indicates that a higher dose level might be a NOEL, these studies are considered as low to medium quality; thus, the data base is given a low confidence rating. The overall confidence in the RfD is low, reflecting the need for more toxicity data by the oral route.

VII. References

ATSDR. 1993. Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Toxicological Profile for Beryllium. TP-92/04. April 1993.

Cotes JE, Gilson JC, McKerrow CB and Oldham P. 1983. A long-term follow-up of workers exposed to beryllium. Br. J. Indust. Med. 40:13-21.

Cullen M, Kominsky J, Rossman M, Cherniack M, Rankin J, Balmes J, Kern J, Daniele R, Palmer L, Naegel G, McManus K, and Cruz R. 1987. Chronic beryllium disease in a precious metal refinery. Clinical epidemiologic and immunologic evidence for continuing risk from exposure to low level beryllium fume. Amer. Rev. Respir. Dis. 135:201-208.

Eisenbud M, and Lisson J. 1983. Epidemiological aspects of beryllium-induced nonmalignant lung disease: A 30 year update. J. Occupat. Med. 25:196-202.

Eisenbud M, Berghout CF, and Steadman L. 1948. Environmental studies in plants and laboratories using beryllium: the acute disease. J. Indust. Hyg. Toxicol. 30: 282-285.

Eisenbud M, Wanta R, Dustan C, Steadman L, Harris W, and Wolf B. 1949. Nonoccupational berylliosis. J. Indust. Hyg. Toxicol. 31:282-294.

Infante PF, Wagoner JK, Sprince NL. 1980. Mortality patterns from lung cancer and nonneoplastic respiratory disease among white males in the beryllium case registry. Environ. Res.. 21:35-43.

Johnson NR. 1983. Beryllium disease among workers in a spacecraft-manufacturing plant - California. MMWR. 32:419-425.

Kriebel D, Sprince NL, Eisen EA, Greaves I, Feldman H and Greene R. 1988a. Beryllium exposure and pulmonary function: A cross sectional study of beryllium workers. British J. Indust. Med. 45:167-173.

Kriebel D, Sprince NL, Eisen EA, and Greaves I. 1988b. Pulmonary function in beryllium workers: Assessment of exposure. Brit. J. Indust. Med. 45:83-92.

Lieben J, and Metzner F. 1959. Epidemiological findings associated with beryllium extraction. Amer. Indust. Hyg. Assoc. 20:494-499.

Metzner F, and Lieben J. 1961. Respiratory disease associated with beryllium disease - A case study. J. Occupat. Med. 3:341-345.

Reeves AL, Deitch D, and Vorwald AJ. 1967. Beryllium carcinogenesis. I. Inhalation exposure of rats to beryllium sulfate aerosol. Cancer Res. 27:439-443.

Reeves AL, Swanborg RH, Busby EK, and Krivanek ND. 1970. In: Target Organ Toxicology Series: Immunotoxicology and Immunopharmacology. Dean J, Luster M, Munson A, and Amos H, eds. (Raven Press, Inc. New York, NY, USA) pp. 441-456.

Schepers GW. 1964. The biological action of beryllium: Reaction of the monkey to inhaled aerosols. Indust. Med. Surg. 33:1-16.

Schepers GW, Durkhan TM, Delahunt AB, and Creedon FT. 1957. The biological action of inhaled beryllium sulfate. A preliminary chronic toxicity study in rats. AMA Arch. Indust. Health 15:32-58.

Schroeder HA, and Mitchner M. 1975. Life-term studies in rats: Effects of aluminum, barium, beryllium and tungsten. J. Nutrition 105:421-427.

Stiefel T, Schulze K, Zorn H, and Toelg G. 1980. Toxicokinetic and toxicodynamic studies of beryllium. Arch. Toxicol. 45:81-92.

Vorwald A, and Reeves A. 1959. Pathologic changes induced by beryllium compounds. Arch. Indust. Health 19:190-199.

Wagner W, Groth D, Holtz J, Madden G, and Stokinger H. 1969. Comparative chronic inhalation toxicity of beryllium ores bertrandite and beryl, with production of pulmonary tumors by beryl. Toxicol. Appl. Pharmacol. 15:10-29.

CHRONIC TOXICITY SUMMARY

1,3-BUTADIENE

(butadiene; buta-1,3-diene; biethylene; bivinyl; vinylethylene)

CAS Registry Number: 106-99-0

I. Chronic Toxicity Summary

Inhalation reference exposure Level	8 μg/m^3
Critical effect(s)	Increased incidence ovarian atrophy in mice
Hazard index target(s)	Reproductive system

II. Physical and Chemical Properties Summary (HSDB, 1995)

Molecular formula	C_4H_6
Molecular weight	54.09 g/mol
Description	Colorless gas
Vapor pressure	910 mm Hg at 20^{o}C
Solubility	Soluble in water (735 mg/L); soluble in ethanol, ether, acetone, benzene
Conversion factor	2.21 mg/m^3 = 1 ppm at 25 ^{o}C

III. Major Uses and Sources

1,3-Butadiene is a major commodity product of the petrochemical industry, usually produced as a by-product of ethylene. The majority of 1,3-butadiene is used in the production of styrene-butadiene rubber copolymers (SBR). Other applications include as a polymer component for polybutadiene, hexamethylene diamine, styrene-butadiene latex, acrylonitrile-butadiene-styrene resins (ABS), chloroprene and nitrile rubbers. A variety of industrial syntheses utilize 1,3-butadiene as a chemical intermediate, such as in the production of adiponitrile (a nylon precursor), captan and captofol fungicides, ethylidene norbornene and sulfolane, boron alkyls, and hexachlorobutadiene. Additionally, 1,3-butadiene has been found in automobile exhaust, gasoline vapor, fossil fuel incineration products, and cigarette smoke (HSDB, 1995).

IV. Effects of Human Exposure

An early occupational study reported complaints of irritation of eyes, nasal passages, throat, and lungs in rubber manufacturing workers following acute exposure to unknown levels of 1,3-butadiene (Wilson, 1944). Additional symptoms reported included coughing, fatigue, and drowsiness, however, all symptoms ceased on removal from the exposure.

Studies on the chronic effects of 1,3-butadiene have been centered in the styrene-butadiene rubber manufacturing industry, which uses large quantities of 1,3-butadiene, and the 1,3-butadiene monomer industry. One retrospective epidemiological study reported a increase in overall mortality, emphysema, and cardiovascular diseases (chronic rheumatic and arteriosclerotic heart disease) among rubber workers (McMichael *et al.*, 1976). Two other occupational studies have described the potential for adverse hematological effects due to butadiene exposure (Checkoway and Williams, 1982; McMichael *et al.*, 1975). A survey of workers at a styrene-butadiene rubber plant revealed slightly lower levels (but within normal range) of red blood cells, hemoglobin, platelets, and neutrophils in exposed (mean 20 ppm) versus unexposed workers (Checkoway and Williams, 1982). And 1,3-butadiene has been implicated in hematopoietic malignancies among styrene-butadiene rubber workers at levels lower than 20 ppm (McMichael *et al.*, 1975). Since the workers in these studies were exposed to mixtures of chemicals, the specific contribution of butadiene to the adverse respiratory and hematopoietic effects remains unclear.

V. Effects of Animal Exposure

The limited number of available chronic animal inhalation studies have focused on the potential carcinogenicity of 1,3-butadiene. The National Toxicology Program (NTP) has sponsored two chronic inhalation studies in $B6C3F_1$ mice (NTP, 1984; Melnick *et al.*, 1990; NTP, 1993), while Hazelton Laboratories Europe (HLE), Ltd, conducted a chronic inhalation study in Sprague-Dawley rats (HLE, 1981; Owen *et al.*, 1987; Owen *et al.*, 1990).

The two $B6C3F_1$ mice inhalation studies sponsored by the National Toxicology Program (Huff et al, 1985; Melnick *et al.*, 1990; NTP, 1984; NTP, 1993), though focused on the carcinogenicity of 1,3-butadiene, identified other adverse chronic effects. The earlier NTP (1984) study in mice administered 0, 625 or 1250 ppm 1,3-butadiene for 6 hours/day, 5 days/week for up to 61 weeks. Nonneoplastic changes observed were elevated testicular and ovarian atrophy at both doses (625 and 1250 ppm); liver necrosis in male mice at both doses and in female mice at 1250 ppm; and, nonneoplastic lesions in the nasal cavity at 1250 ppm. At this highest dose, adverse changes found in the nasal cavity included chronic inflammation, fibrosis, cartilaginous metaplasia, osseous metaplasia, and atrophy of the sensory epithelium. No nasal or respiratory lesions were seen in the controls. This study identified a chronic LOAEL of 625 ppm for gonadal atrophy in both sexes.

The later NTP study (Melnick *et al.*, 1990; NTP, 1993) used lower exposure concentrations of 1,3-butadiene (0, 6.25, 20, 62.5, 200 or 625 ppm) administered 6 hours/day, 5 days/week for up to 2 years. Two-year survival was significantly decreased in mice exposed to 20 ppm and greater, primarily due to chemical-related malignant neoplasms. Increased incidences of nonneoplastic lesions in exposed mice included bone marrow atrophy, gonadal atrophy (testicular, ovarian and uterine), angiectasis, alveolar epithelial hyperplasia, forestomach epithelial hyperplasia and cardiac endothelial hyperplasia. Gonadal atrophy was observed at 200 ppm and 625 ppm for males and at 6.25 ppm and higher for females. Bone marrow toxicity

(regenerative anemia) was seen at 62.5 ppm and higher. This study identified a chronic LOAEL of 6.25 ppm for reproductive toxicity, and a NOAEL of 200 ppm and a LOAEL of 625 for nonneoplastic hematotoxic effects.

The U.S. EPA (1985) reviewed data from a 2-year chronic inhalation toxicity study sponsored by the International Institute of Synthetic Rubber Producers (IISRP) at Hazelton Laboratories Europe, Ltd (1981) on Sprague-Dawley rats exposed to 0, 1000 or 8000 ppm 1,3-butadiene. Results from the study were also reported later by Owen *et al.* (1987; 1990). Minor clinical effects, including excessive eye and nose secretions plus slight ataxia, were observed between 2 and 5 months in rats exposed to 8000 ppm 1,3-butadiene. Alterations in organ weight were also observed in this high exposure group; a dose-related increase in liver weights was observed at both the 52-week interim kill and at study termination. Absolute and relative kidney weight was also significantly increased and associated with nephrosis. No reproductive organ atrophy was reported in this rat study.

The U.S. EPA (1985) described another secondary report, that of Miller (1978), which reviewed a group of Russian studies of subchronic 1,3-butadiene exposure in rats. One study discussed (Ripp, 1967) continuously exposed rats to relatively lower concentrations, 0.45, 1.4 or 13.5 ppm. At 13.5 ppm, blood cholinesterase was elevated, blood pressure was lowered, and motor activity was decreased. Histopathological changes reported at 0.45 ppm were congestion in the spleen and hyperemia and leukocyte infiltration of cardiac tissue. Alterations in lung tissue noted at 1.4 and 13.5 ppm included atelectasis, interstitial pneumonia, and emphysema. No other studies utilize such low exposure levels or measured such endpoints. Unfortunately, the specific research methods and results for this study are unavailable for direct review and comparison.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	NTP (1993)
Study population	$B6C3F_1$ mice (70/sex/group)
Exposure method	Discontinuous inhalation (0,6.25,20,62.5,200,625 ppm) over 2 years
Critical Effects	Increased incidence of ovarian atrophy
Exposure continuity	6 hr/d, 5 d/wk
Exposure duration	103 weeks
LOAEL	6.3 ppm
NOAEL	Not observed
Average experimental exposure	1.1 ppm for LOAEL group
Human equivalent concentration	1.1 ppm for LOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
Subchronic factor	1
LOAEL uncertainty factor	10
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10

Cumulative uncertainty factor	300
Inhalation reference exposure level	4 ppb (0.004 ppm; 0.008 mg/m^3; 8 µg/m^3)

Significant reproductive toxicity was observed in both sexes of mice at the interim 9-month, interim 15-month, and 2-year study termination as gonadal atrophy (NTP, 1993). Testicular atrophy was induced in male B6C3F1 mice at 625 ppm or above in this principal study and in a previous study (NTP, 1984). In female mice exposed for 9-months, ovarian atrophy was observed at 200 and 625 ppm (442 or 1381 mg/m^3, respectively). After 15 months, ovarian atrophy was observed at exposure levels of 20 ppm (44.2 mg/m^3) and above. In mice exposed for up to 2 years (103 weeks), the incidence of ovarian atrophy increased at all exposure concentrations relative to controls, establishing a chronic LOAEL of 6.25 ppm (13.81 mg/m^3) for reproductive toxicity.

Few chronic animal studies are available for comparison, however, an acute and subchronic (10 week) study identified male-mediated F1 effects in mice exposed to 12.5 or 1250 ppm 1,3-butadiene (6 hour/day, 5 days/week) (Anderson *et al.*, 1993). At 1250 ppm (2762.4 mg/m^3), statistically significant effects observed were a reduction in the number of implantations, an induction of dominant lethal mutations, an increased incidence of early and late deaths, and an increase in abnormalities. The lower dose, 12.5 ppm (27.63 mg/m^3), resulted in an increase of early deaths and fetal abnormalities. The IISRP sponsored study (Owen *et al.* 1987; 1991) did not report any noncancer adverse reproductive effects in Sprague-Dawley rats exposed to 1000 or 8000 ppm 1,3-butadiene (2210 or 17680 mg/m^3, respectively); however, tumors were found in reproductive tissues (Owen *et al.*, 1987).

The major strength of the butadiene REL is the observation of a dose-response effect in a well-conducted lifetime inhalation exposure study. The major weaknesses are the lack of adequate human health effects data and the lack of a NOAEL observation.

VII. References

Anderson D, Edwards AJ, Brinkworth MH. 1993. Male-mediated F1 effects in mice exposed to 1,3-butadiene. In *Butadiene and Styrene: Assessments of Health Hazards.* (Sorsa, M, Peltonen K, Vainio H, Hemminki K, Eds.) IARC Scientific Publications No. 127. pp. 171-181. International Agency for Research on Cancer, Lyon, France.

Checkoway H, Williams TM. 1982. A hematology survey of workers at a styrene-butadiene synthetic rubber manufacturing plant. Am Ind Hyg Assoc J 43(3):164-169.

HLE. 1981. Hazelton Laboratories Europe, Ltd. 1,3-Butadiene: The toxicity and carcinogenicity of butadiene gas administered to rats by inhalation for approximately 24 months. Prepared for the International Institute of Synthetic Rubber Producers, New York, NY. Unpublished. As reported in U.S. EPA, 1985.

HSDB. 1995. Hazardous Substances Data Bank. National Library of Medicine, Bethesda, Maryland (CD-ROM Version). Micromedex Inc., Denver, Colorado (Edition expires).

Huff JE, Melnick RL, Solleveld HA, Haseman JK, Powers M, and Miller RA. 1985. Multiple organ carcinogenicity of 1,3-butadiene in B6C3F1 mice after 60 weeks of inhalation exposure. Science 227(4686):548-549.

McMichael AJ, Spirtas R, and Kupper KK. 1975. Solvent exposure and leukemia among rubber workers: An epidemiologic study. J Occup Med 17:234-239.

McMichael AJ, Spirtas, R, Gamble JF, and Tousey PM. 1976. Mortality among rubber workers. Relationship to specific jobs. J Occup Med 18(3)178-185.

Melnick RL, Huff J, Chou BJ, and Miller R. 1990. Carcinogenicity of 1,3-butadiene in C57BL/6xC3H F1 mice at low exposure concentrations. Cancer Res 50(20):6592-6599.

Miller LM. 1978. Investigation of selected potential environmental contaminants: Butadiene and its oligomers. Philadelphia, PA: Franklin Research Center. As reported in U.S. EPA, 1985.

NTP. 1984. U.S. National Toxicology Program. *Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No.106-99-0) in B6C3F1 Mice (Inhalation Studies)* (TR No.288)Research Triangle Park, NC, National Institute of Environmental Health Sciences.

NTP. 1993. U.S. National Toxicology Program. *Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No.106-99-0) in B6C3F1 Mice (Inhalation Studies)* (TR No.434)Research Triangle Park, NC, National Institute of Environmental Health Sciences.

Owen PE, Glaister JR, Gaunt IF, and Pullinger DH. 1987. Inhalation toxicity studies with 1,3-butadiene. 3. Two-year toxicity/carcinogenicity studies in rats. Am Ind Hyg Assoc J 48:407-413.

Owen PE, and Glaister JR. 1990. Inhalation Toxicity and Carcinogenicity of 1,3-Butadiene in Sprague-Dawley Rats. Environ Health Persp 86:18-25.

U.S. EPA. 1985. U.S. Environmental Protection Agency. *Mutagenicity and Carcinogenicity Assessment of 1,3-Butadiene*. Washington, DC: U.S. Environmental Protection Agency, Office of Health and Environmental Assessment. Publication No. EPA/600/8-85/004F.

Wilson RH. 1944. Health hazards encountered in the manufacture of synthetic rubber. J Am Med Assoc 124(11):701-703.